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Behavioural outcomes and neuropathology associated with mild traumatic brain injury

Behavioural outcomes and neuropathology associated with mild traumatic brain injury

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Abstract

Mild traumatic brain injury (mTBI) is an injury to the head caused by external force or blunt trauma that leads to an alteration in consciousness; loss of consciousness of less than 30 minutes and post-traumatic amnesia of less than 24 hours may be present. Previous studies have suggested there is an association between mTBI sustained in youth and risk behaviours. Additionally, MRI techniques that assess brain microstructure have been shown to be sensitive to the neuropathology of mTBI.

I carried out a systematic review to explore the association between mTBI in youth and later risk behaviour. This highlighted a paucity of good quality longitudinal evidence. I then conducted a study investigating the same association using data from ALSPAC, a large longitudinal birth cohort. I included a negative exposure control group of participants with orthopaedic injury (OI) to uncover potentially unmeasured confounding factors. Using logistic and ordinal regression on outcomes related to substance use, crime and psychiatric symptoms, I found causal evidence for an association between mTBI and hazardous alcohol use.

Next, I explored the association between mTBI and four MRI-based measures of the cortex in a subsample of ALSPAC participants. Unexpectedly, OI was associated with higher values for some of the measures, suggesting a potential bias in the data. Finally, I carried out a diffusion tensor imaging study on rugby players who had recently sustained a sport-related mTBI and found indications of possible oedema or axonal injury in the recently injured group. This study was limited by a small sample size and wide range of time post-injury, however.

My findings highlight the importance of providing support to young people following mTBI and continued consideration of mTBI in high-contact sports. Future research should explore the link between mTBI, alcohol use and neuropathology using longitudinal research, negative control design and multiple MRI methods.

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I am very grateful to all the participants of ALSPAC and the ALSPAC team for making a lot of this research possible, and to the University of Bristol men's rugby club for collaborating with me. In particular, thanks to the first team coach, Joe Goodman, and the club physiotherapist, Jane West, for sending participants my way, and to all the players who took part. Thank you to all the staff at CRiC for their help with the rugby study. I'd also like to extend my gratitude to the University of Bristol and the IEU for funding my studentship.

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Author’s Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE: .....

Publications

Kennedy, E., Cohen, M., & Munafò, M. (2017). Childhood traumatic brain injury and the associations with risk behaviour in adolescence and young adulthood: a systematic review. *The Journal of Head Trauma Rehabilitation*, 32(6), 425.

Kennedy, E., Heron, J., & Munafò, M. (2017). Substance use, criminal behaviour and psychiatric symptoms following childhood traumatic brain injury: findings from the ALSPAC cohort. *European Child & Adolescent Psychiatry*, 26(10), 1197-1206.

These publications were adapted to form Chapters 2 and 3, as well as sections in Chapter 1. Miriam Cohen performed a ten percent data check on the systematic review; Jon Heron provided advice on statistical methods for the ALSPAC study; Marcus Munafò provided guidance and feedback on study design and manuscript preparation for both publications.

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Glossary of Abbreviations

AD	Axial diffusivity
ADHD	Attention deficit hyperactivity disorder
ALSPAC	Avon longitudinal study of parents and children
AUDIT	Alcohol use disorders identification test
CAST	Cannabis abuse screening test
CD	Conduct disorder
CHDS	Christchurch health and development study
CI	Confidence Interval
CTE	Chronic traumatic encephalopathy
DAWBA	Development and well-being assessment
DSM	Diagnostic statistical manual of mental disorders
DTI	Diffusion tensor imaging
EEG	Electroencephalogram
FA	Fractional anisotropy
FOV	Field of view
FSL	Oxford centre for functional mri of the brain software library
FTND	Fagerström test for nicotine dependence
GCS	Glasgow coma scale
GM	Grey matter
GRADE	The grading of recommendations assessment, development and evaluation
GWAS	Genome-wide association study
ICBM	International consortium for brain mapping
ICD	International classification of diseases
IQ	Intelligence quotient
IRR	Increased relative risk
LOC	Loss of consciousness
MD	Mean diffusivity
MNI	Montreal Neurological Institute
MP-RAGE	Magnetisation prepared rapid acquisition gradient echo
MRI	Magnetic resonance imaging
MT	Magnetisation transfer

MTBI	Mild traumatic brain injury
MTR	Magnetisation transfer ratio
MWF	Myelin water fraction
NART	National adult reading test
NFL	National football league
ODD	Oppositional defiant disorder
OI	Orthopaedic injury
OR	Odds ratio
PCS	Post concussive symptoms
PO	Proportional odds
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
PTA	Post-traumatic amnesia
RD	Radial diffusivity
RF	Radio frequency
RFU	Rugby football union
SD	Standard deviation
SDQ	Strengths and difficulties questionnaire
SLF	Superior longitudinal fasciculus
SPGR	Spoiled gradient recalled
TBI	Traumatic brain injury
TBSS	Tract-based spatial statistics
TE	Echo time
TFCE	Threshold-free cluster enhancement
TMT	Trail-making task
TR	Repetition time
UK	United Kingdom
WISC	Wechsler intelligence scale for children
WM	White matter

Chapter 1 Introduction

1.1 Overview of Mild Traumatic Brain Injury

1.1.1 Prevalence of TBI

Traumatic brain injury (TBI) is the leading cause of death and disability in children and young adults globally (WHO, 2006). It affects over one million people in the United States every year (Langlois, Rutland-Brown, & Wald, 2006) and occurs at an annual rate of approximately 235 per 100,000 in Europe (Tagliaferri, Compagnone, Korsic, Servadei, & Kraus, 2006). Incidence rates are generally based on medical records, so the rate is presumed to be higher when unreported injuries are considered (Cassidy et al., 2004). Mild TBIs (hereafter mTBI) account for between 70% and 90% of all treated cases and are more common among adolescents and young adults (Cassidy et al., 2004). A recent review of paediatric TBI estimated that each year TBI affects over 3 million children worldwide, and that over 80% of these are mild (Dewan, Mummareddy, Wellons, & Bonfield, 2016). The term mTBI is synonymous with the term concussion; the latter is more commonly used in the context of sport-related head trauma and is derived from the Latin *'concutere'* meaning "to dash together or shake". There is some debate about the usefulness and appropriateness of the term concussion (for a review see (Sharp & Jenkins, 2015)).

1.1.2 Definition of mTBI

Severity of a TBI can be based on the score on the Glasgow Coma Scale (GCS) (Jennett & Teasdale, 1977), the length of loss of consciousness (LOC) and/or the length of post-traumatic amnesia (PTA). The GCS is an assessment of the eye,

verbal and motor responses of a patient; the scale ranges from 3 to 15, with a lower score indicating a more severe injury. Based on the GCS, a score between 13 to 15 classifies a mTBI. For a TBI to be classified as mild, LOC should not exceed 30 minutes, nor should PTA exceed 24 hours if either symptom is present. However, LOC and PTA are not necessary requirements for a diagnosis of mTBI, while a perfect GCS score of 15 does not confer any information about a patient, and two patients with a GCS score of 15 may present quite differently. A mTBI may also have occurred if there is an alteration of mental state such as feelings of being dazed, disoriented or confused (Kay et al., 1993; Menon, Schwab, Wright, & Maas, 2010).

Box 1.1 Conceptual Definition of mTBI from the Centers for Disease Control and Prevention (Center for Injury Prevention and Control, 2003).

MTBI is an injury to the head (arising from blunt trauma or acceleration or deceleration forces) that results in one or more of the following:

- *any period of confusion, disorientation, or impaired consciousness;*
- *any dysfunction of memory around the time of injury;*
- *loss of consciousness lasting less than 30 minutes*
- *the onset of observed signs or symptoms of neurological or neuropsychological dysfunction, including*:
  - Seizures acutely following injury to the head;
  - Irritability, lethargy, or vomiting following head injury, especially among infants and very young children
  - Headache, dizziness, irritability, fatigue, or poor concentration, especially among older children and adults.

(Center for Injury Prevention and Control, 2003).

1.1.3 Post-Concussive Symptoms

The constellation of symptoms that are associated with a mTBI are known as post-concussive symptoms (PCS). These include physical symptoms like headache, dizziness, nausea, vomiting, vertigo and fatigue or difficulty sleeping; cognitive symptoms such as difficulty concentrating, lack of attention and memory problems; and psychological symptoms such as irritability and feelings of depression or frustration (King, Crawford, Wenden, Moss, & Wade, 1995; Sharp & Jenkins, 2015). For most individuals with a mTBI these symptoms will resolve from 1 week to 3 months, however in approximately 15% of cases some symptoms will persist (Signoretti, Vagnozzi, Tavazzi, & Lazzarino, 2010).

There is some debate about the reliability and usefulness of post-concussive symptoms as an indicator of mTBI. The symptoms have a high baseline prevalence in the general population and have considerable overlap with other disorders such as depression and post-traumatic stress disorder (Rapp & Curley, 2012). Nonetheless current treatment and management of mTBI is based on the resolution of PCS. Individuals who have sustained a mTBI are advised to abstain from both physical and cognitive activities until these symptoms subside.

1.2 OBSERVATIONAL STUDIES OF RISK BEHAVIOUR AND MTBI

1.2.1 Observational Studies

Studying outcomes related to TBI necessitates the use of observational (rather than experimental) data. Randomised controlled trials provide the most robust evidence for a causal association (Gage, Munafò, & Davey Smith, 2016). In a randomised controlled trial, participants are randomly assigned to be in an

exposure condition and followed up to assess the outcomes that occur in each condition. When the exposure of interest is something that may cause harm to an individual it is unethical to manipulate this exposure through experimental means. In other words, this approach is not feasible in human research into mTBI as it would be unethical to inflict a head trauma on one group of individuals while sparing a comparable group for evaluation. Instead, observational methods must be used for casual inference; both cross-sectional and longitudinal observational research has been employed to explore associations with mTBI.

In observational research an association observed between the outcome and exposure could be due to a causal relationship between the two, but confounding, reverse causation and bias are also possibilities. Confounding can be partly overcome by adjusting for all potential confounders in the analyses, but there is still the potential for residual confounding from unmeasured factors and measurement error. Reverse causation is difficult to overcome, as the causal direction is hard to establish using observational methods. Using data where there is a temporal gap between the exposure and outcome helps, but it is still possible that pre-existing outcomes that influence the exposure could lead to the observed associations. Different types of bias, such as recall bias and selection bias, can affect results. Both biases can be reduced by careful recruitment of participants and controls into the study. Recall bias can be reduced by limiting the timeframe of recalling events (i.e., asking about events "in the past 12 months" rather than "in your lifetime").

1.2.1.1 Cross sectional Research

In cross-sectional research a selection of the population participates in a study and is asked about their exposure and outcome status. This approach is relatively inexpensive and can be useful for establishing associations. However, reverse causality cannot be ruled out using cross-sectional research as there is no indication of a temporal sequence. Additionally, recall bias can be an issue if the question is inaccurately phrased or if the timeframe of events is too broad; for example, simply asking if a participant "has ever had a concussion" without asking about some symptoms of mTBI or including a definition may preclude those who do not know what level of head trauma may constitute a mTBI, or those who sustained a mTBI many years previously.

1.2.1.2 Case-Control Studies

Case-control studies include a sample of individuals with a diagnosis of interest and a sample of individuals without that diagnosis. This type of design is often used to assess outcomes of mTBI, particularly in neuroimaging research. A smaller sample size can be used as the number of cases are already determined by the recruitment process. There is more certainty that an individual has experienced a mTBI as participants can be recruited based on diagnosis of a mTBI by a medical professional. The use of appropriate control participants is vital to reduce confounding and selection bias.

1.2.1.3 Cohort Studies

Cohort studies are particularly well placed to explore causality of mTBI. The term 'cohort' has military etymology; in the Roman army a legion was made up of ten cohorts of soldiers, with 300-600 individuals in each (Grimes & Schulz, 2002). In research, however a cohort study is a longitudinal approach where a

specific group of people are assessed over time to track the progression of disease. Synonyms for these studies include longitudinal study, prospective study or follow-up study. Birth cohort studies are one type of cohort study where, as the name suggests, data are collected on a group of individuals across their lifetime beginning at birth. These types of studies are particularly well suited to studying causal factors and outcomes of mTBI in childhood and adolescence, where the participant can be seen to be free from mTBI at the onset of the study and information was collected both prior to and following the injury. However, longitudinal research has limitations including selection bias, loss to follow up and they are quite expensive studies to run.

1.2.1.4 Observational data in this thesis

In observational research, an association observed between the outcome and exposure could indicate a causal association or it could be due to confounding, reverse causation or bias (Gage et al., 2016). In this thesis I have used data from a longitudinal birth cohort study in Chapters 3 and 4. Longitudinal studies provide the strongest evidence for causal inference as there is a temporal relationship between exposure and outcome, and in birth cohort studies information about exposures can be reported in close proximity to when it happened, minimising the issue of recall bias. In order to strengthen causal inference further, I incorporated a negative control exposure group, where confounding structures are likely to be similar but there is no pathway between the exposure and the outcome (Rees, 2003). Negative control designs are employed to uncover potentially unmeasured confounding or bias by comparing the main analysis of interest to a second analysis between the negative control exposure and main outcome, see Figure 1.1 for a schematic representation. If there

is an association of larger magnitude between the exposure of interest and the outcome, then it adds to the strength of evidence for a causal association. The negative control chosen must have no plausible biological mechanism for the association with the outcome of interest and have a similar confounding structure to the outcome of interest (Gage et al., 2016).

In Chapters 3 and 4, I have included a group of participants with orthopaedic injuries (OI) as well as an uninjured control group. There is no evidence that sustaining a fracture or broken bone disrupts neural processes in the same way a mTBI could, so there is no plausible biological mechanism linking OI to my outcomes of interest. However, it may be more accurate to say that there is no *obvious* biological mechanism as it is feasible that the altered range of motion experienced during the recovery process from an OI could lead to a change in brain structure. For example, Draganski and colleagues found transient alterations in grey matter in participants who learned to juggle compared to a non-juggling control group, these structural changes were temporary as they were not observed following a break from juggling (Draganski et al., 2004). No similar investigation of structural brain changes following OI has been carried out.

Nonetheless, including an extracranial injury group is also intended to adjust for unmeasured confounding factors related to sustaining an injury such as the effect of being involved in an accident, receiving medical attention, missing school and recovering from the injury. Any associations found between my outcomes of interest and the OI group will encourage closer examination of potential biases and confounding that might influence associations between mTBI and the same outcomes.

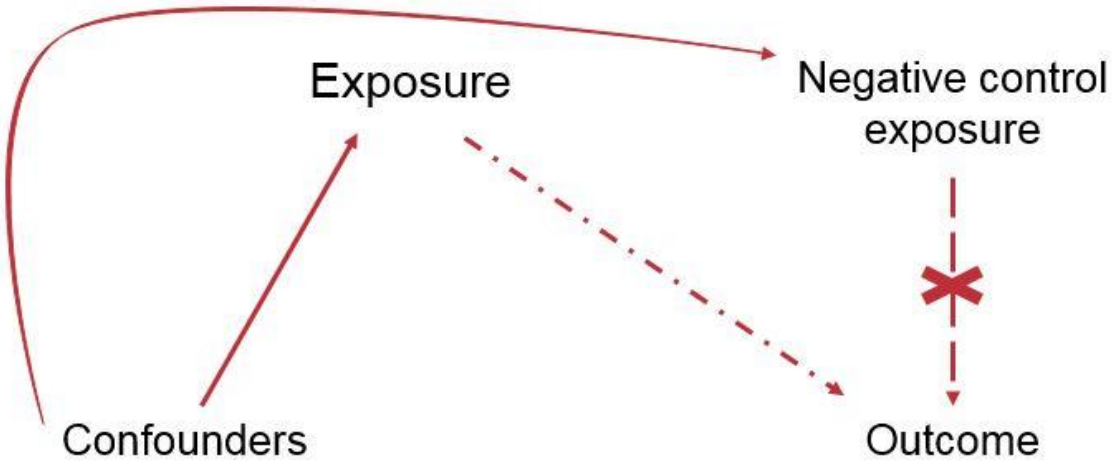

Figure 1-1 Schematic representation of negative control exposure. Confounding is the same for the exposure of interest and the negative control exposure. There is no causal association between the negative control exposure and the outcome of interest. The dashed-and-dotted line represents the main association under investigation; the dashed line represents the negative control analysis. Figure adapted from Gage et al., 2016.

1.2.2 MTBI in childhood and adolescence

Although a peak in function recovery within the first six to twelve months following a childhood TBI is often reported (Chapman, 2007), longer term effects of a childhood TBI may not become apparent until later developmental stages, when more complex demands are placed on an individual (Taylor & Alden, 1997). Adolescence is a time of increased demand as an individual transitions to relative independence, and enhanced social cognitive skills are required to navigate increasingly intricate and intimate relationships (Blakemore & Mills, 2014). An increase in risk-taking behaviour is also typically seen in adolescence, Steinberg argues that the heightened salience of peer relations in adolescence is key to the increased risk-taking behaviour seen at this age (Steinberg, 2008). Chein and colleagues (Chein, Albert, Brien, Uckert, & Steinberg, 2011) report that the presence of peers increases the number of risks taken by adolescents in a

simulation driving task. In a functional magnetic resonance imaging (fMRI) task, adolescents being observed by peers had greater activation of reward-related brain regions, including the ventral striatum and the orbitofrontal cortex, than the two older age groups (Chein et al., 2011).

Hessen and colleagues carried out a follow-up study in patients admitted to hospital for a mTBI; 45 people who were injured before age 15 years and 74 injured after age 15 years completed a comprehensive assessment of neuropsychological function 23 years after their index injury. The authors found that all participants had mean test scores within the normal range (Hessen, Nestvold, & Anderson, 2007). However, in the group injured during childhood, mTBI with post-traumatic amnesia (PTA) lasting over 30 minutes or PTA of over 30 minutes in combination with a pathological EEG within 24 hours was strongly predictive of poor neuropsychological outcome. This was not the case for adults with the same diagnostic variables, which the authors suggested was indicative of greater vulnerability in children to the long-term consequences of complicated mTBI than adults.

1.2.3 Risk behaviour and mTBI in youth

Previous cross-sectional research has shown increased substance use (Ilie et al., 2015), disruptive behaviour disorders (Max et al., 1998), school violence (Ilie et al., 2016) and conduct problems (Ilie et al., 2014; Tonks, Williams, Yates, & Slater, 2011) in participants with a history of mTBI. Tonks and colleagues (Tonks et al., 2011) found higher parent and teacher ratings of social difficulties at age 10 to 16 years in participants who had experienced a TBI approximately four years previous, and also for participants aged 8 to 10 years for whom a TBI event

occurred between birth and age 5 years. When compared to orthopaedic injury controls, 8 to 13 year old participants with a severe TBI that occurred 12 to 63 months previously had poorer communication and social skills, but this was not the case for participants with a mTBI (Robinson et al., 2014). A Canadian study of high-school children, aged 13 to 20 years, assessed the relationship between TBI and substance use in over 6,000 participants using a cross-sectional survey design; a subsample of over 3,000 participants also completed questionnaires about substance-related problems, hazardous alcohol use and problematic cannabis use. TBI in this sample was defined as a self-reported head injury that resulted in at least a five minute loss of consciousness or one overnight hospital stay, this was correlated with concurrent items relating to medically treated injuries, which indicated that participants with a history of TBI had an average 2-fold increase in substance use in the past 12 months (adjusted odds ratios ranged from 1.87 for binge drinking to 3.77 for methamphetamine use). In the subsample assessed for substance use problems, those with a TBI history were at increased risk for problems relating to alcohol and cannabis use as well as substance-related risks as measured by the CRAFFT Screening Tool (CRAFFT is a mnemonic acronym relating to key words in each item; Car, Relax, Alone, Forget, Friends and Trouble). However, the study did not provide information on participant age at the time or severity of the injury (Ilie et al., 2015).

Evidence for a prospective association between mTBI and negative behavioural outcomes comes from three cohort studies. In a sample of over one million Swedish people, having a TBI registered with hospital before age 25 years was associated with increased risk of adverse outcomes, including drawing a disability pension, psychiatric visit or hospitalisation, low educational attainment,

and welfare recipiency. For those with a mTBI, the risk ratios for these outcomes ranged from 1.18 to 1.52 (Sariaslan, Sharp, Onofrio, Larsson, & Fazel, 2016). Findings from the Northern Finland 1966 Birth Cohort Study indicate that a mTBI before age 14 years was associated with drinking to intoxication at age 14 years (Winqvist, Jokelainen, Luukinen, & Hillbom, 2007). In the same cohort, male participants with a TBI before age 15 years were at higher risk of committing a crime registered with the Ministry of Justice from ages 16 to 31 years, and those with a TBI had a 2-fold increased risk of developing a psychiatric disorder, which increased to 4-fold for criminality combined with a psychiatric disorder (Timonen et al., 2002). In the Christchurch Health and Development Study (CHDS), participants who had experienced a mTBI requiring an inpatient hospital stay between birth and age five years had higher self- and parent-ratings of conduct disorder/oppositional defiant disorder and substance abuse at age 14 to 16 years (McKinlay, Grace, Horwood, Fergusson, & MacFarlane, 2009), and a higher likelihood of alcohol and drug dependence at age 16 to 25 years, which mediated a relationship between the same injury and an increased number of arrests, property offenses and violent offenses (McKinlay, Corrigan, Horwood, & Fergusson, 2014). Any mTBI at age 6 to 15 years was linked with increased arrests and property offenses at age 16 to 25 years, hospitalisation for the injury was additionally associated with violent offenses (McKinlay et al., 2014).

In this thesis I explored the association between mTBI and later risk behaviour in Chapters 2 and 3 using systematic review and epidemiological analysis of birth cohort data respectively.

1.3 NEUROPATHOLOGY

1.3.1 Neuroimaging

Computed tomography (CT) scans conducted at the time of injury often show no neuropathology in mTBI, nor do conventional magnetic resonance imaging (MRI) techniques. On the other hand, MRI techniques that assess microstructural properties may be more sensitive to neuropathology in mTBI (Bigler, 2013).

1.3.1.1 Neuroanatomy and neuroimaging terms

Before beginning a discussion of neuroimaging techniques used in mTBI research, it may be helpful to provide a brief overview of neuroanatomy and common neuroimaging terms. There are four primary lobes that make up the cortex of the brain (see Figure 1.2). Anterior refers to regions located towards the front of the brain, posterior refers to regions towards the back of the brain. Dorsal refers to regions that are located towards the top of the brain, ventral refers to regions that are located towards the base of the brain. Superior refers to regions 'above', while inferior refers to regions 'below'. Similarly, regions situated towards the midline of the brain, where the two hemispheres meet, are said to be medial and regions situated away from the midline and more towards the edges of the brain are referred to as lateral.

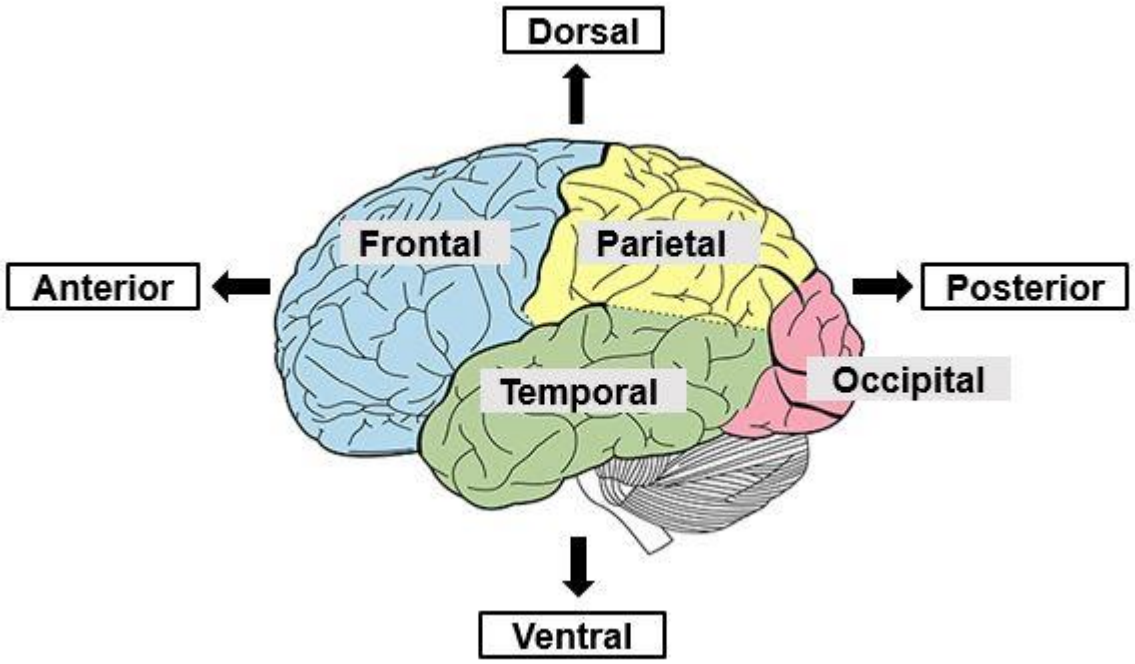

Figure 1-2 Basic neuroanatomy and neuroimaging terms

The human brain contains both grey and white matter. Myelin, a lipid-protein lamellar membranous structure that covers axons, is what makes white matter appear white. The human brain contains approximately 176, 000 km of myelinated axons (Paus, 2010) which connect different regions of the brain. Myelin acts as an electrical insulator for neurons and the speed of conduction is 10 to 100 times faster in myelinated compared to unmyelinated axons, which is fundamental in allowing complex neuronal functions to occur. MRI techniques that provide an index of myelin do so through measurement of water molecules in and around the myelinated axons (Laule et al., 2007). Figure 1.3 below shows a central nervous system (CNS) myelin sheath with a close up of the bilayer and proteins contained therein.

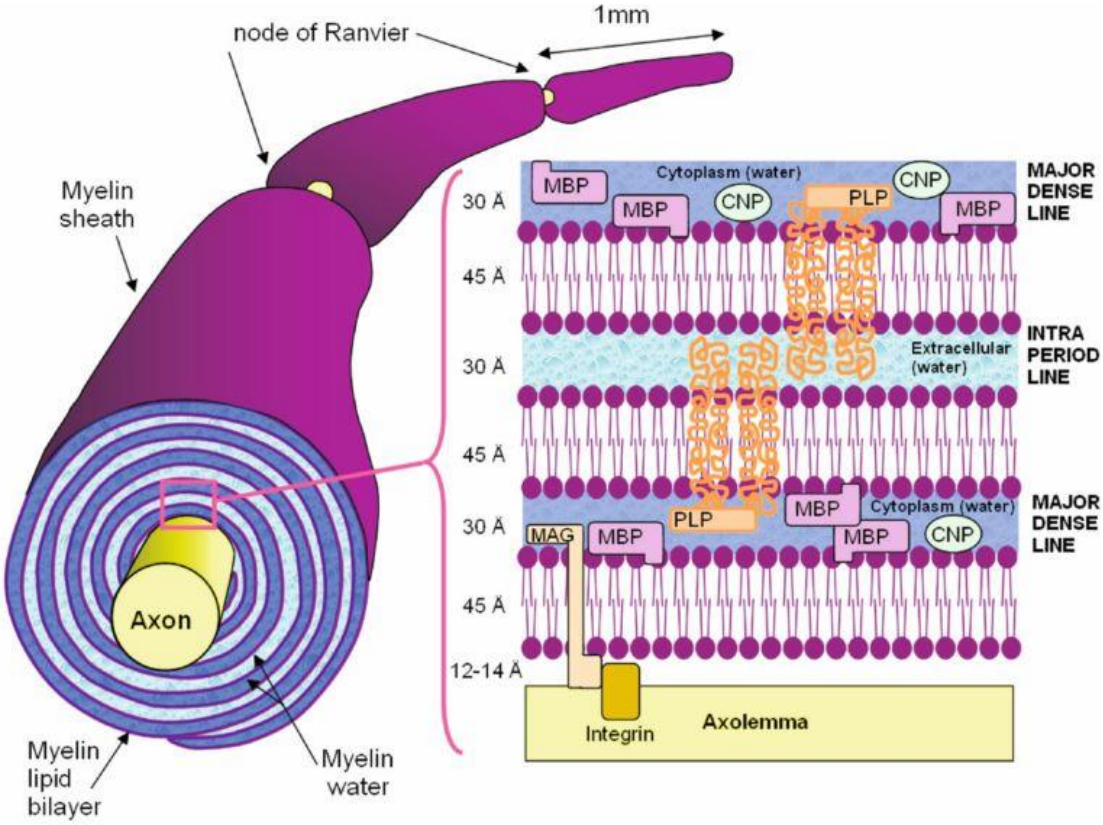

Figure 1-3 The central nervous system myelin sheath surrounding an axon with a close up of the bilayer depicted. Proteins in the bilayer include myelin basic protein (MBP), proteolipid protein (PLP), cyclic nucleotide phosphodiesterase (CNP) and myelin-associated glycoprotein (MAG). This diagram is taken from Laule et al., (2007).

1.3.1.2 Structural MRI

The human body contains mostly water (H_2O), the protons of which align when placed in a magnetic field. In an MRI machine, the magnet creates the B0 field along the z axis and when a participant is positioned in an MRI machine the H_2O protons in the body align to that field. The scanner applies a radio frequency (RF) pulse to knock the alignment of the protons into the x-y plane. When this RF is removed, the protons gradually return to their normal spin, aligned with the magnetic field z axis, this process releases a radio signal which is used to create the image. The time required for the majority of the spin to return to being parallel with the z axis of the MRI (i.e. net magnetisation to return to 63% of its maximum

value) is called T1 relaxation time. Structural images are created using T1 relaxation time as different tissue types have different T1 relaxation times. An associated measure is T2 relaxation time which is a measure of the dephasing of the protons in the x-y plane (i.e. when the transverse magnetisation falls to about 37% of its initial value following the RF pulse). Both T1 and T2 relaxation time are measured in milliseconds.

1.3.1.3 MRI techniques used in mTBI research

Diffusion tensor imaging (DTI) is a neuroimaging technique that provides a measure of white-matter microstructure. This technique is described in more detail in Chapter 5, but briefly DTI is based on the diffusion of water molecules restricted by the myelin sheaths and cell membranes of white matter tracts. In white matter tracts, the fibres are oriented in the same direction; water molecules diffuse faster parallel to the long axis of a fibre bundle and slower perpendicular to the fibres. This characteristic is anisotropic diffusion and is indexed by fractional anisotropy (FA), which approximates the shape of the ellipse created by the ratio of the speed of water molecules moving parallel and perpendicular to the long axis of the fibre bundle. FA ranges in value from 0-1. A very simple explanation is that low FA score can reflect white matter degradation as the axon membranes may no longer be able to constrain water diffusion, while elevated FA can reflect a restriction of water diffusion due to oedema (Bigler, 2013). However, caution is necessary when considering such simple explanations as FA is also determined by the organisation and packing of fibres.

Myelin water fraction (MWF) is an index of water between the layers of myelin derived from quantitative T2 relaxation. Quantitative measurement of T2

relaxation can distinguish three distinct water environments in the brain: cerebrospinal fluid has a relaxation rate of approximated 2 seconds; intra- and extracellular water has a rate of approximately 80 milliseconds while water between myelin bilayers has a rate of approximately 20 milliseconds (Alonso-Ortiz, Levesque, & Pike, 2015). MWF has a close correlation with histological studies (i.e. studies of brain tissue samples) and has helped to clarify the interpretation of FA values. Mädler and colleagues compared MWF from T2 relaxometry with FA in healthy participants (Mädler, Drabycz, Kolind, Whittall, & Mackay, 2008) and overall found a strong positive correlation between FA and MWF; however, this differed in some regions. For example, the forceps major contains multiple fibre crossings and disorganised fibre bundles; therefore, it has a low FA but in fact a high degree of myelin content. Conversely, highly organised structures such as the genu of the corpus callosum have a high FA, but this does not correspond to equally high myelin content.

Another parameter that has been used to investigate myelin integrity following mTBI is the magnetisation transfer ratio (MTR). MTR is the percent signal change between one acquisition with a magnetisation transfer (MT) saturation pulse and an acquisition without an MT pulse. MT is the physical process by which macromolecules and their closely associated water molecules (the "bound" pool) cross-relax with protons in the free water pool (Laule et al., 2007). Macromolecules are molecules containing a very large number of atoms, such as a protein; the macromolecules of myelin are the dominant source of the MT signal in white matter. MTR is a good measure of tissue damage but is influenced by processes such as inflammation, axonal density and the presence of microphages (Laule et al., 2007). In simplistic terms, lower MTR indicates less

myelin for example Grossman suggests that in multiple sclerosis (a disorder characterised by demyelination in the central nervous system) it could be said that slightly lower MTR suggests inflammation, whereas much lower MTR represents demyelination (Grossman, Gomori, Ramer, Lexa, & Schnall, 1994).

1.3.2 Pathophysiology

The pathophysiology of TBI is believed to be a two-stage process. The primary mechanical stage is an immediate result of the external force that impacts the brain; this leads to mechanical damage such as contusion, laceration and intracranial haemorrhage (Signoretti et al., 2010; Xiong, Mahmood, & Chopp, 2013). The secondary stage is delayed and is the result of a complex chemical cascade induced by the shear forces of the primary injury. In this later stage, neuronal depolarisation induces a surge in glutamate which leads to a huge influx of calcium, which further results in mitochondria damage, increased formation of free radicals and increased expression of chemokines and cytokines. The brain damage that may result from the secondary injury includes cell death, axonal damage, demyelination and brain atrophy (Xiong et al., 2013). Damage extending to the ascending reticular pathway is the suggested cause of LOC; while damage involving the limbic connections sub-serving the Papez circuit is the suggested cause of PTA (Rees, 2003).

The response to mTBI in the developing brain is different to the mature brain (Choe, Babikian, Difiori, Hovda, & Giza, 2012). Unmyelinated axons show more vulnerability to the effects of TBI; as the myelination process is ongoing in the developing brain fibers may be more susceptible to axonal injury. Importantly, neural plasticity during brain development is influenced GABA-mediated

inhibition and this can be disrupted by the impaired neural activation that happens following mTBI. Managing paediatric mTBI should involve engaging in moderate activity to stimulate neural circuitry to promote brain recovery, while minimizing the risk of additional injury during recovery (Choe et al., 2012).

1.3.3 Neuroimaging Findings mTBI

Most of the MRI studies of mTBI have used DTI; there is evidence of differences in FA between participants with mTBI and control participants (Aoki & Inokuchi, 2016; Aoki, Inokuchi, Gunshin, Yahagi, & Suwa, 2012). In a recent meta-analysis of 21 studies that used a whole-brain voxel-based approach, Aoki and Inokuchi identified three brain regions with lower FA in mTBI participants relative to controls (Aoki & Inokuchi, 2016). The largest cluster extended from the left thalamus to the splenium of the corpus callosum; the second cluster was in the left forceps minor and the third was in the right superior longitudinal fasciculus. This supported an earlier meta-analysis by the same authors looking at regions of interest studies where lower FA in the splenium and midbody of the corpus callosum was identified in mTBI (Aoki et al., 2012).

However, studies investigating mTBI and white matter using DTI have been somewhat equivocal in terms of lower or higher FA; this lack of consistency could be due to differences such as time between injury and assessment or the sample included. In a study on 22 athletes with a history of concussion, Churchill and colleagues reported higher FA in the corona radiata and genu of the corpus callosum relative to athletes without prior concussion when assessed an average of 26 months since most recent concussion (Churchill et al., 2017b). Inglese and colleagues found lower FA in the splenium of the corpus callosum and the

internal capsule in a group of participants who were 4 days post-injury and a group of participants that were 5.7 years post-injury relative to a control group (Inglese et al., 2005). Elsewhere, participants from the military who had sustained a blast-related mTBI with loss of consciousness had a greater number of clusters of reduced FA throughout the brain compared with those without TBI history (Miller, Hayes, Lafleche, Salat, & Verfaellie, 2016).

Only one study has used MWF in the mTBI literature; Wright and colleagues scanned 45 college ice hockey players before the season began and then scanned those who sustained a concussion within 72 hours of the injury, and again two weeks and two months post-injury (Wright et al., 2016). Eleven players sustained a concussion and their follow-up scans were compared to their baseline scan. The authors found reduced MWF in voxel clusters at 2 weeks post-injury in the splenium of the corpus callosum, the right posterior thalamic radiation, the left superior corona radiata, left superior longitudinal fasciculus and left posterior limb of the internal capsule. At 2 months post-injury there was no longer any evidence of decreased MWF. No differences were seen for pre- and post-season in athletes who had not sustained a concussion. The authors suggest that the transient change in MWF could indicate that myelin fragmentation and degeneration occurred in the acute phase of injury but remyelination of the affected axons had occurred by two months post injury (Wright et al., 2016).

Three studies have utilised MT imaging. In a study of 13 patients with persistent PCS, 2 patients had MTR in the splenium of the corpus callosum that was 2 SDs below a comparison group of ten healthy controls; the grouped average was lower in the mTBI group also (McGowan et al., 2000). However, Narayana and colleagues assessed 56 patients with mTBI and 54 patients with orthopaedic

injuries and found no differences in MTR on a scan conducted approximately 24 hours post-injury; and no differences within-subjects between baseline scans and scans acquired approximately 90 days post-injury (Narayana et al., 2015). In an earlier study, the curve width of the MTR histogram of segmented white matter was found to be sensitive to post-concussion symptoms in 13 participants with mTBI relative to age- and gender-matched controls. However, the authors note the limited sensitivity and specificity of the measure to PCS (Hofman, Kemerink, Jolles, & Wilmink, 1999).

1.4 Summary and Aims

MTBI is an injury to the head caused by external force that results in an alteration of consciousness, with LOC less than 30 minutes and PTA of less than 24 hours if either symptom is present. Post-concussive symptoms following a mTBI include physical and cognitive difficulties. However, the usefulness of these symptoms has been debated as there is a high prevalence of these symptoms in the general population and they are associated other disorders such as depression (Rapp & Curley, 2012). Nonetheless there is evidence that suggests mTBI in youth is associated with negative behavioural outcomes (Sariaslan et al., 2016). Furthermore, MRI techniques that assess the microstructural properties of the brain have provided evidence for differences in the brain structure of individuals who have sustained a mTBI relative to those who have not (Aoki & Inokuchi, 2016).

There are two main aims of this thesis. First, I aim to expand on the knowledge of long-term negative behavioural outcomes associated with mTBI in

childhood and adolescence. This will be achieved through a systematic review of the existing evidence in Chapter 2 and through a study using longitudinal data and a negative control design in Chapter 3. Second, I aim to better characterise the neuropathology of mTBI using MRI techniques that assess microstructural properties. In Chapter 4, I will use MRI data from the same longitudinal study to look at differences in brain microstructure of participants with and without a history of mTBI. Finally, Chapter 5 also speaks to this second aim as I present findings from a case-control DTI study of rugby players who have recently sustained a sport-related mTBI. The analyses in Chapters 2 and 3 are exploratory. While in Chapters 4 and 5, I expect there will be differences between the participants with mTBI and the control groups in the MRI measures assessed. However, I have no predictions regarding the direction of effect.

Chapter 2 Childhood TBI and the Associations with Risk Behaviour in Adolescence and Young Adulthood: A Systematic Review

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A version of this systematic review has been published in the Journal of Head Trauma Rehabilitation: Kennedy, E., Cohen, M., & Munafò, M. (2017). Childhood traumatic brain injury and the associations with risk behaviour in adolescence and young adulthood: a systematic review. *The Journal of Head Trauma Rehabilitation*, 32(6), 425.

2.1 BACKGROUND

As described in Chapter 1 (Section 1.2.3), there is evidence from both cross-sectional (Ilie et al., 2014, 2015; Tonks et al., 2011) and longitudinal research (McKinlay et al., 2014, 2009; Sariaslan et al., 2016; Winqvist et al., 2007) suggesting an association between TBI in youth and negative behavioural outcomes. The aim of this chapter was to systematically review the TBI literature in order to provide a clearer picture of the relationship between childhood TBI and risk behaviour in adolescence. Risk behaviour was defined as any use of alcohol, tobacco or illicit substances, behavioural issues of conduct or involvement in criminal activity. The review was exploratory in nature with the aims of clarifying any relationship that exists and highlighting any patterns of association, such as the role of age at TBI event.

2.2 METHOD

2.2.1 Literature Search

The review was carried out according to the PRISMA guidelines (www.prisma-statment.org). Electronic databases (PubMed and Web of Science) were searched until the end of March 2015 to identify English-language studies exploring the association between childhood traumatic brain injury and risk behaviour in adolescence and young adulthood. The following search terms were used: *((((child*) OR (pediatric)) AND (traumatic brain or brain or head injury)) AND ((adolescen*) AND ((psychosocial or antisocial or conduct*) OR (substance ??use))) NOT (animal) NOT (adult))*. At the first stage of the filtering process, titles were excluded if there was no mention of TBI or head injury; abstracts were excluded if the outcomes clearly did not relate to the risk behaviours. Following exclusion of irrelevant articles based on title and abstract, the remaining studies were screened, and references sections were hand-checked for any additional suitable articles.

Studies were included if they detailed: 1) original research, 2) were written in English, 3) used a case control or longitudinal design, 4) reported the TBI event to have occurred between birth and 13 years of age, and 5) assessed the outcome over 13 years. Review articles, intervention studies and reports of non-impact related brain damage (e.g., stroke or brain tumour) were excluded from the review. The cut-off age of 13 years was chosen to differentiate between childhood and adolescence as well as to ensure the outcome behaviours were being adequately measured, for example it is uncommon for substance use to be assessed before this age.

Data were extracted on: the location and design of the study, the age of the participants at injury and assessment, the identification, definition and classification of TBI, the measures used to assess outcomes, and any covariates considered in analysis. All stages of the review were conducted by me; a ten percent check was carried out by a colleague (MC in the published manuscript), which indicated that no studies were excluded that should have been included.

2.3 RESULTS

2.3.1 Characterisation of Studies

The initial search yielded 2,209 articles, excluding duplicates. Fourteen journal articles were chosen for full text review, following which eight were excluded, see Figure 2.1 for the PRISMA flow diagram. Six journal articles were reviewed, which were based on four separate studies. Two articles were based on a New Zealand longitudinal study, and two on an Australian longitudinal study, while the other two were from the United Kingdom and Finland.

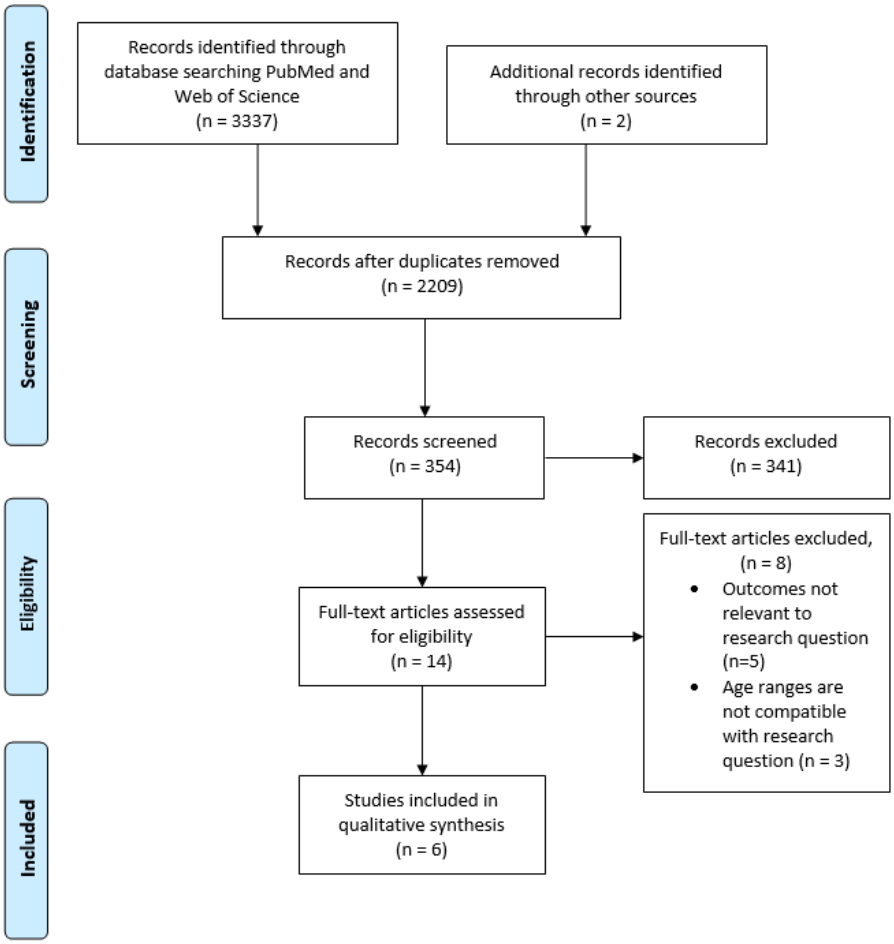

Figure 2-1 PRISMA Flow Diagram

2.3.2 Summary of Results

This section describes the findings of each study based on study design. Articles from the same country and cohort will be summarised in the same section. Figures relating to the study findings are presented in Table 2.1 due to inconsistencies in reporting across the six studies. Excluded studies are shown in Table 2.2.

2.3.2.1 Cross-sectional

Only one of the articles included in the current review used a cross-sectional design, the UK study by Tonks and colleagues (Tonks et al., 2011). This study was based on a cohort of participants recruited from occupational therapy services compared to an age- and gender-matched group of controls. The parents and teachers of the children, who were aged between 10 and 16 years at the time of assessment, completed the Strengths and Difficulties Questionnaire (Goodman, 1997) to investigate emotional difficulties in the participants. Both parent and teacher ratings of conduct disorders, peer problems and negative impact of behaviour in the home environment for the TBI participants were higher than for no injury controls.

2.3.2.2 Longitudinal

The remaining five articles each used a longitudinal design; three articles utilised data from two separate birth cohort studies (McKinlay et al., 2014, 2009; Winqvist et al., 2007), while the other two articles used the same follow up data from hospital admissions (Rosema et al., 2014b, 2015).

Winqvist and colleagues utilised the Northern Finland 1966 Birth Cohort, which encompasses 96% of births in the northern provinces of Finland in that year for a total of 12,058 children (Winqvist et al., 2007). Participants were grouped in terms of TBI and no TBI history based on the Finnish Hospital Discharge Register up to age 14 years. The severity of the injuries was based on the International Classification of Diseases 8th revision. At age 14 years, all participants were asked if they had ever drunk alcohol and if so, if they had ever been drunk. The TBI group were more likely to report drinking to intoxication. Factors associated with

drinking to intoxication were having a mTBI, coming from a one-parent family, having an urban residence and parental alcohol misuse.

Two of the included articles (McKinlay et al., 2014, 2009) were based on the Christchurch Health and Development Study (CHDS), an epidemiological birth cohort from New Zealand, which includes 1,265 births from mid-1977. Data were gathered at birth, at 4 months and at annual intervals until age 16 years and again at ages 18, 21 and 25 years. Information was garnered from a combination of self-report, parent interview, teacher questionnaire, medical records and other official records (McKinlay et al., 2008).

In both articles, the authors focussed on mTBI grouped as 'inpatient TBI' and 'outpatient TBI'. The former were admitted to hospital for two days or less, while the latter were seen by a general practitioner or at an accident and emergency department then sent home. For the TBI to be classified as mild there had to have been a loss of consciousness of no more than 20 minutes; post traumatic amnesia of less than 2 hours if present; no neurological signs and no evidence of skull fracture and a Glasgow Coma Scale (Jennett & Teasdale, 1977) (GCS) score of more than 14. Both groups were compared to an uninjured control group in analyses.

In the first of the two CHDS studies (McKinlay et al., 2009), information was gathered at ages 14 to 16 years on conduct disorder/oppositional defiant disorder (CD/ODD) and alcohol or illicit substance use/dependence using mother and self-report scales. Children who experienced an inpatient TBI between the ages of birth and 5 years had an increased likelihood of a CD/ODD DSM-III-R diagnosis; this remained evident when maternal punitiveness at age 3 and family

adverse life events were adjusted for. Likewise, inpatient TBI increased the odds of having a diagnosis of alcohol or illicit substance use/dependence at age 14 to 16 years, which remained once covariates were adjusted for.

In a later study (McKinlay et al., 2014), data were collated from self-report measures on alcohol dependence, drug dependence, number of arrests, property offenses and violent offenses between the ages of 16 to 25 years. Analyses also adjusted for the individual's gender, family socioeconomic status at the child's birth, early behaviour problems and parental substance abuse/dependence. Experiencing an inpatient TBI between birth and age 5 years increased the likelihood of alcohol dependence and drug dependence. Inpatient TBI also increased the number of arrests; property offenses and violent offenses. The outpatient TBI group had an increased risk of violent offending. However, when alcohol and drug dependence were added as covariates, the increased risk of arrests, property offenses and violent offenses were no longer supported in either group injured before age 5 years.

Participants for the remaining two articles were recruited from hospital admissions to the Royal Children's Hospital in Melbourne (Rosema et al., 2014b, 2015). The GCS (Jennett & Teasdale, 1977) was used to classify the severity of the injury and a control group of uninjured children were selected from preschools and childcare centres. The participants were aged between 1 year and 7 years 11 months at the time of the injury and both studies explored outcomes 16 years after the event.

The Adult Self-Report (Achenbach & Rescorla, 2003) was used to explore externalising behaviour problems, in the first study comparing participants who

had experienced a TBI to those who had not (Rosema et al., 2014b). No differences were found between the groups on self-reports of overall externalising behaviour, aggression, or rule-breaking behaviour.

In another study of the same cohort (Rosema et al., 2015), the Adult Behavior Checklist (Achenbach & Rescorla, 2003) (completed by parents) revealed no differences in externalising symptoms, between mTBI, moderate TBI, severe TBI and no TBI groups.

Table 2-1 Summary of Findings from Included Studies

Paper	**Country**	**Participants' Age (years)**	**Sample Size;** **Grouping**	**Outcomes**
McKinlay et al., 2014 (McKinlay et al., 2014)	New Zealand	Age at injury: 0 -5 Age at assessment: 16 - 25	N= 953 - 1055 Inpatient mTBI: n = 22 Outpatient mTBI: n = 55 - 61 No injury: n = 876 - 972	Substance Use: Inpatient Alcohol OR 2.46, 95% Cl 0.94 to 6.71, p < .10 Outpatient Alcohol OR 1.54, 95% Cl 0.75 to 3.12, p = n.s. Inpatient Drug OR 2.85, 95% Cl 1.11 to 7.32, p < .05 Outpatient Drug OR 1.24, 95% Cl 0.60 to 1.28, p = n.s. Behaviour: Inpatient Arrests IRR 4.33, 95% Cl 2.55 to 7.34, p < .01 Outpatient Arrests IRR 1.36, 95% Cl 0.86 to 2.13, p = n.s. Inpatient Property offenses IRR 2.24, 95% Cl 1.42 to 3.52, p < .01 Outpatient Property offenses IRR 1.35, 95% Cl 0.99 to 1.84, p < .10 Inpatient Violent offenses IRR 2.72, 95% Cl 1.74 to 4.26, p < .01 Outpatient Violent offense IRR 1.47, 95% Cl 1.08 to 1.99, p < .05
McKinlay et al., 2009 (McKinlay et al., 2009)	New Zealand	Age at injury: 0 -5 Age at assessment: 14 - 16	N = 915 Inpatient mTBI: n = 19 Outpatient mTBI: n = 57 No injury: n = 839	Substance Use: Inpatient OR 3.1, 95% Cl 1.1 to 8.5, p < .05 Behaviour: Inpatient Conduct Disorder/Oppositional Defiant Disorder OR 4.9 (1.8 to 13.4), p < .01

Rosema et al., 2015 (Rosema et al., 2015)	Australia	Age at injury: 1 - 8 Age at assessment: 17 - 23	N = 104 MTBI: n = 13 Moderate TBI: n = 40 Severe TBI: n = 22 No TBI: n = 29	Behaviour: Externalising behaviour p = 0.67
Rosema et al., 2014 (Rosema et al., 2014b)	Australia	Age at injury: 1 - 8 Age at assessment: mean 21.47	N = 54 TBI: n = 36 No TBI: n = 18	Behaviour: Externalising behaviour p = 0.57 Aggression p = 0.36 Rule-Breaking behaviour p = 0.46
Tonks et al., 2011 (Tonks et al., 2011)	United Kingdom	Age at injury: 3.7 years before assessment Age at assessment: 10 - 16	N = 81 TBI: n = 14 No TBI: n = 67	Behaviour: Conduct problems p < .01 Peer problems p < .01
Winqvist et al., 2007(Winqvist et al., 2007)	Finland	Age at injury: 0 - 14 Age at assessment: 14	N = 10281 TBI: n = 176 No TBI: n = 10105	Substance Use: Drinking to intoxication p < .01

Table 2-2 Excluded Studies

Paper	Country	Reason for Exclusion
Anderson et al., 2012 (Anderson et al., 2012)	Australia	Outcomes not relevant
DeMatteo et al., 2014 (DeMatteo et al., 2014)	USA	Age range at injury too wide
Donders & Strom, 2000 (Donders & Strom, 2000)	USA	Outcomes not relevant
Green et al., 2013 (Green et al., 2013)	Australia	Outcomes not relevant
McKinlay et al., 2002 (McKinlay et al., 2002)	New Zealand	Age at outcome too young
Muscara et al., 2009(Muscara et al., 2009)	Australia	Outcomes not relevant
Rosema et al., 2014 (Rosema et al., 2014a)	Australia	Outcomes not relevant
Timonen et al., 2002 (Timonen et al., 2002)	Finland	Age range too wide

2.3.3 Quality of evidence

All of the included studies were observational and therefore initially rated as having low quality of evidence based on GRADE criteria.

2.3.3.1 Cross-sectional

The quality of evidence for the study by Tonks and colleagues (Tonks et al., 2011) was downgraded to very low as there was no consideration of confounding and no effect sizes were reported. Nevertheless, participants were recruited appropriately, and controls were matched for age and gender.

2.3.3.2 Longitudinal

The study by Winqvist and colleagues (Winqvist et al., 2007) had a low quality of evidence. There was good consideration of confounding and a moderate effect size with a reasonable confidence interval. The effect size was not large enough to increase the overall quality of evidence. Strengths of this study include the large sample of TBI participants identified from a hospital register with appropriate uninjured controls.

The McKinlay and colleagues (McKinlay et al., 2014, 2009) studies had a low quality of evidence. The consideration of confounding was very good, although the confidence intervals were too wide to increase the quality to moderate. The large sample size and inclusion of an uninjured matched control group were strengths.

The Rosema and colleagues (Rosema et al., 2014b, 2015) study had a very low quality of evidence. There were no effect sizes or confidence intervals reported. In one article there was no consideration of confounding, while in the other socioeconomic status was included as the only covariate. The sample size was small, particularly for the control groups.

2.4 DISCUSSION

The aim of this chapter was to explore the association between childhood TBI and risk behaviour in adolescence and young adulthood. Six articles based on four studies were identified: two birth cohort studies, one longitudinal follow-up study and one cross sectional study. Five of the articles assessed problematic behaviour as an outcome of early life TBI, while substance use was an outcome in three articles. All of the studies compared participants with a history of TBI to

participants without a TBI. In all three articles exploring substance use, a positive relationship was found between mTBI and substance use (McKinlay et al., 2014, 2009; Winqvist et al., 2007). Findings relating to behavioural issues were less consistent across the five articles, the TBI groups in three of the articles had poorer behavioural outcomes (McKinlay et al., 2014, 2009; Tonks et al., 2011), while there were no differences between groups in the remaining two articles (Rosema et al., 2014b, 2015).

The quality of evidence for all four studies ranged from low to very low, in part due the observational design of the studies. The cross-sectional study (Tonks et al., 2011) and the prospective longitudinal study (Rosema et al., 2014b, 2015) were downgraded to a very low quality of evidence as neither study adequately controlled for plausible confounding, and both had relatively small sample sizes. Additionally, the study by Tonks and colleagues (Tonks et al., 2011) reported neither effect size estimates nor confidence intervals. Both of the birth cohort studies (McKinlay et al., 2014, 2009; Winqvist et al., 2007) were rated as providing low quality of evidence; notably, plausible confounding was taken into consideration and the sample sizes were large. There was some indication of a dose-response relationship between injury severity and the outcomes of interest, but this differed between the two studies; Winqvist and colleagues (Winqvist et al., 2007) found an association with mTBI and drinking to intoxication, while McKinlay and colleagues (McKinlay et al., 2014, 2009) found that a certain threshold of mTBI was necessary for an association to be seen. The effect sizes and confidence intervals were not of great enough magnitude in either study to increase the quality of evidence rating from low to moderate.

A considerable strength of the included articles is the use of medical records to identify and classify TBI, and also the consistency of the use of the GCS across three of the four included studies (the GCS was unavailable when injury was assessed in the Northern Finland 1966 Birth Cohort (Winqvist et al., 2007)). However the TBI groups were variously formed based on severity in terms of mild versus moderate-to-severe (Rosema et al., 2015; Winqvist et al., 2007), severity of a mTBI (McKinlay et al., 2014, 2009) or the presence of a TBI (Rosema et al., 2014b; Tonks et al., 2011), which makes comparison more difficult. Additionally, there is some question about the sensitivity of the GCS to measure milder injuries; for example, Rees argued that a maximum score of 15 does not help in determining whether a brain injury has occurred. Three of the articles found relationships between risk behaviour and mTBI; however, the severity was classified differently. Winqvist and colleagues classed participants as having a mTBI based on ICD 8th Revision codes corresponding to concussion and skull fractures (Winqvist et al., 2007); however it is unclear whether the inclusion of skull fractures could be more in keeping with the 'complicated mild' severity put forward by Williams and colleagues who found neurobehavioural outcome at 6 months was comparable to moderate injury when the mTBI included a depressed skull fracture or brain lesion (Williams, Levin, & Eisenberg, 1990). Conversely McKinlay and colleagues excluded participants from the mTBI group if there was evidence of a skull fracture, and used loss of consciousness of less than 20 minutes as one signifier of a mild injury (McKinlay et al., 2014, 2009). This length of time is in keeping with a recent report for the Children's Commission where a mild injury was defined as a LOC of between 10 and 20 minutes (Hughes, Williams, Chitsabesan, Davies, & Mounce, 2012); however the

American Congress of Rehabilitation Medicine suggests a loss of consciousness of up to 30 minutes is still a mTBI (Kay et al., 1993). There is a need for clarification and harmonisation across studies. One important caveat is that relying on medical records alone may misrepresent the prevalence of TBI; higher rates of self-reported TBI compared to rates obtained through medical records suggest that not all those who incur a TBI will present to medical services (Hughes et al., 2012). This may be particular pertinent if, for example, the TBI was sustained in the context of illegal activity.

The control groups in all of the included studies were age-matched participants without a history of TBI. It has been argued that an additional trauma group should be included in studies of TBI to control for factors associated with injury that may be poorly measured (Dikmen, Ross, Machamer, & Temkin, 1995). Rees reviewed five articles that assessed persistent post-concussive syndrome in mTBI and in non-brain related injuries and reported comparable outcomes between both groups (Rees, 2003). In a study of post-injury substance use among participants with a TBI and a spinal cord injury, Kolakowsky-Hayner and colleagues reported no differences in drinking patterns and higher rates of illicit drug use in participants with a spinal cord injury than those with a TBI (Kolakowsky-Hayner et al., 2002). Satz has recommended that in order to confirm a head injury rather than a general injury effect, a difference between a head injury and other injury group as well as a difference between a head injury and no injury group must be observed (Satz, 2001). To control for injury factors, such as pain experience or posttraumatic stress (Rees, 2003), future research should aim to include an extracranial injury group alongside an uninjured control group to act as a negative control.

2.4.1 Limitations

The evidence presented in this chapter indicates that the associations between childhood TBI and later risk behaviour are not yet understood. However, there are some limitations to this review. First, the literature search yielded a rather small set of articles based on four unique participant samples. One possibility is that the exclusion of non-English language publications may have resulted in some articles being missed. No librarian was involved in the search strategy, which may have been beneficial. However, the low number of studies may simply indicate a paucity of research on the long-term outcomes of childhood TBI on risk behaviour. Second, it was not possible to carry out a quantitative synthesis (i.e., meta-analysis) on the results because of the variety of outcomes assessed and the differences in TBI groupings. For example, within three articles, substance use was measured in terms of drinking alcohol to intoxication (Winqvist et al., 2007), through survey questions (McKinlay et al., 2009) or by use of the Composite International Diagnostic Interview (McKinlay et al., 2014). Third, while the quality of evidence for observational studies is rated as low by the GRADE approach, two of the included studies were downgraded to very low. This makes it more difficult to draw firm conclusions and could be avoided in future by adjusting for all potential confounders and clearly reporting effect sizes and confidences intervals.

2.4.1 Chapter summary

From the articles reviewed here, it is difficult to draw any clear conclusions about the relationship between TBI in childhood and adolescence and later risk behaviour. Although there is some support for a link between mTBI in

early life and later substance use (McKinlay et al., 2014, 2009; Winqvist et al., 2007), more research is necessary. In the next chapter, I will build on these articles using data from a birth cohort study. To maintain the quality of evidence, effect sizes and confidence intervals are clearly reported. Furthermore, I have incorporated a negative control design including participants with an orthopaedic injury as well as an uninjured control group – this will control for general injury effects and unmeasured confounding as well as increasing the ability to infer a causal interpretation.

Chapter 3 Substance Use, Criminal Behaviour and Psychiatric Symptoms following mTBI

The findings from this chapter have been published in European Child and Adolescent Psychiatry: Kennedy, E., Heron, J., & Munafò, M. (2017). Substance use, criminal behaviour and psychiatric symptoms following childhood traumatic brain injury: findings from the ALSPAC cohort. *European Child & Adolescent Psychiatry*, 26(10), 1197-1206.

3.1 BACKGROUND

The evidence from the systematic review presented in Chapter 2 highlighted a paucity of high quality longitudinal research investigating TBI in youth and later risk behaviour and suggested that more research is needed to draw any strong conclusions about this relationship. There was some evidence for an association between mTBI in youth and increased substance use in adolescence and young adulthood (McKinlay et al., 2014, 2009; Winqvist et al., 2007).

In this chapter, I investigated the association between mTBI and risk behaviour in a United Kingdom birth cohort. MTBI was based on incidences of skull fracture and loss of consciousness due to a head injury reported by parents and children at multiple time points up to age 16 years. Risk behaviour was defined as psychiatric symptoms, substance use, and criminal behaviours. In order to strengthen causal inference, I included a negative control exposure group, where confounding structures are likely to be similar but there is no pathway between the exposure and the outcome (Rees, 2003). If the observed association is larger for exposure of interest than for the negative control exposure this increases

confidence that the association may be causal (Gage et al., 2016). Previously, in a Swedish population study, individuals who sustained non-TBI fall-related injuries were less likely to have poor adult outcomes than those with a TBI before age 25 years (Sariaslan et al., 2016). Additionally, Fazel and colleagues found that participants with a history of epilepsy were less likely to commit violent crime than those who sustained a TBI (Fazel, Lichtenstein, Grann, & Langstrom, 2011). In this study, participants with a history of fracture or broken bone formed the negative control exposure group as this type of injury has a similar confounding structure to TBI but lacks a plausible biological mechanism (i.e., brain injury) for a causal effect on risk behaviour. The effect of age at injury was investigated in secondary analyses separating the cohort into those with childhood injuries and those with adolescent injuries.

3.2 METHOD

3.2.1 Participants

Participants were drawn from a longitudinal birth cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC). Initially 14,541 pregnant women who were expected to give birth between 1 April 1991 and 31 December 1992 were recruited into the study in the South West region of England (Boyd et al., 2012). The study website contains details of all data available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

3.2.2 Measures

3.2.2.1 Injury Groups.

In the ALSPAC questionnaires, parents were asked if their child had incurred any injuries across a range of ages up to 11 years. There were five parents' questionnaires with overlapping age ranges at time of injury. For example, at offspring age 4 years parents were asked about injuries since born; then at offspring age 5 years, parents were asked about injuries since age 4.5 years; the next two questionnaires (offspring age 6 and 8 years) asked parents about four different age ranges, including from birth to 2 years and 3 to 4 years; in the final parent questionnaire, at offspring age 11 years, there was one item related to the same timeframe, namely injuries from birth to age 4 years.

Similar self-report questionnaires were completed by the offspring; at age 15 years participants reported on fractures incurred since their 12th birthday, including skull fractures, and at age 16 years participants reported on a head injury since their 14th birthday or fractures in the last 6 months. Information was gathered from a series of postal questionnaires. A positive response to the item "head injury resulting in a loss of consciousness" or the item "cracked or broke skull" was used to identify participants in the mTBI group. A positive response to any of the items "broke arm or hand", "broke leg or foot" or "broken other bone" was used to identify participants in the orthopaedic injury (OI) control group. Participants who experienced both a head injury and a broken bone were included in the TBI group only. Participants for whom there were no positive responses to the above items were included in the no injury control group. For the secondary analyses, participants were assigned to either a childhood (between birth and age

11 years) and adolescent (between age 12 and 16 years) injury group based on the age at which their first injury occurred.

3.2.2.2 Substance Use.

Data on tobacco, alcohol and cannabis use was gathered by a self-report questionnaire at age 17 years. Problematic use was assessed at age 17 years using the Fagerström Test for Nicotine Dependence (FTND) (Heatherton, Kozlowski, Frecker, & Fagerström, 1991), Alcohol Use Disorders Identification Test (AUDIT) (Babor, Higgins-Biddle, Saunders, & Monteiro, 2001), and the Cannabis Abuse Screening Test (CAST) (Legleye, Piontek, Kraus, Morand, & Falissard, 2013). Responses were used to create category variables for each substance. The FTND is a six-item scale with total scores ranging from 0 to 10; the tobacco variable contained the levels: "not regular smoker", "weekly smoker" and "FTND score of over 4". The AUDIT consists of ten items with total score ranging from 0 to 40, I used a cut-off score of 8 to identify hazardous drinkers. The alcohol use variable contained the levels "non-hazardous use" and "hazardous use". The CAST is a four-item scale with a total score range from 0 to 6; cannabis use was categorised as "not used in the last 12 months", "used in the last 12 months" and "CAST score of one or more". Conservative cut-off scores were used to define problematic use to reflect the young age of the participants.

3.2.2.3 Criminal Behaviour.

A self-report questionnaire at age 17 years was used to assess criminal behaviour in terms of offences committed and trouble with the police (Cho et al., 2015). Participants were classified as either having committed "no offences", "at

least one non-violent offence" or "at least one violent offence" based on questions relating to behaviours such as theft, assault and property damage. There was a single item asking if the participant had "sold illegal drugs to someone" within this questionnaire. A second variable related to whether or not a participant had ever been in trouble with the police was included with the levels "never", "in trouble with the police with no conviction" and "one or more criminal record offence".

3.2.2.4 Psychiatric Symptoms.

Parents completed one measure of psychiatric symptoms, the Strengths and Difficulties Questionnaire (SDQ), while the offspring completed the Development and Well-Being Assessment (DAWBA). The SDQ (Goodman, 1997) is a 25-item parent-rated scale; each item can be rated as 'not true', 'somewhat true' or 'certainly true'. There are ten strengths, fourteen difficulties and one neutral item within five subscales. Parents completed the entire SDQ; however, only two of the subscales assessing conduct problems and peer problems at age 16 years were included in the current analysis. The DAWBA (Goodman, Heiervang, Collishaw, & Goodman, 2011) is a semi-structured interview administered to the offspring at age 15 years. The interview contains sections measuring symptoms of various emotional, behavioural and hyperactivity disorders with skip-rules. The questions are designed to closely follow the diagnostic criteria for psychiatric disorders as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) or the International Classification of Diseases (ICD-10). A composite variable of externalising disorder symptoms, as well as individual variables relating to diagnoses of

oppositional defiant disorder (ODD), conduct disorder (CD) and attention deficit hyperactivity disorder (ADHD) at age 15 years were included as variables in the secondary analysis on childhood injuries.

3.2.2.5 Confounders.

Models were adjusted for confounders that preceded the TBI measurements and were previously shown to have associations with TBI. Confounders considered included: (a) pre-birth confounders (mother's age and education at birth (McKinlay et al., 2010), social class (based on either the paternal or maternal self-reported highest occupation level related to the Registrar General's classification of occupations) and gender), and (b) childhood confounders (early life events (McKinlay et al., 2010), parenting style (based on self-report questionnaires assessing parental bonding, positive and negative parenting experiences) (McKinlay et al., 2010), maternal alcohol use (Winqvist et al., 2007) and maternal tobacco smoking). Tobacco, alcohol and cannabis were mutually adjusted for by including these variables as covariates in the final adjustment model

3.2.3 Statistical Analysis

Ordinal regression was used to explore the association between childhood injuries from birth to age 16 years, and the three-level variables relating to substance use (tobacco and cannabis) and criminal behaviour (offences, trouble with the police) at age 17 years. The gologit2 command (Williams, 2006) was used to permit testing for the more parsimonious proportional odds model (PO). I first, for the univariable model consisting of outcome and exposure, compared

constrained (PO) and unconstrained (non-PO) models using a likelihood ratio test, accepting the simpler model if the p-value was greater than .01. Next, confounders were included without the PO restriction for these additional covariates. Finally, support for PO for the exposure was re-examined within these multivariable models.

Logistic regression was used to explore the association between childhood injuries and the two-level variables relating to substance use (alcohol) and psychiatric symptoms. Separate secondary analyses were conducted using childhood injuries, sustained between birth and age 11 years, and adolescent injuries, sustained between age 12 and age 16, to explore the impact of age at injury.

The impact of confounders on the relationship between TBI and risk behaviours was explored by comparing unadjusted estimates with those adjusted for pre-birth variables (model 1) and those further adjusted for childhood variables (model 2). Substance use and crime frequently co-occur. To explore the impact this relationship may have on the main association of interest, an additional model adjusted for other substance use variables (model 3) was conducted for analyses of each of the substance use and crime variables. This model included adjustment for crime variables in the analyses on substance use. As each level of adjustment increases, the sample size decreases as those with missing data are excluded from the analysis. As a sensitivity analysis, all analyses were conducted on the full sample and then conducted on only those participants with complete data (i.e., complete cases). Comparisons were made between the no injury controls and each injury group, and also directly between the TBI group and the OI group. For the comparison between the TBI and OI groups, additional

sensitivity analyses were conducted excluding participants who had incurred both a TBI and OI. Analyses were conducted using Stata version 13 (StataCorp LP, Texas).

3.3 RESULTS

3.3.1 Characteristics of Participants

Descriptive statistics for the sample are shown in Table 3.1 and a flow chart of the final sample in Figure 3.1. Between birth and age 16 years there were 800 participants with a reported TBI (57% male), 2,305 participants with a reported OI (56% male) and 8,307 participants with neither injury reported (50% male). There were 289 participants included in the TBI groups who had incurred both a TBI and an OI. There were 56 participants who experienced more than one TBI. Participants with a TBI were more likely to be male and to have more adverse early life events. Unexpectedly, individuals with no reported injury were more likely to come from a low-income family and to live in rented subsidised housing; their mothers had a lower level of education and were on average six months younger than mothers of children with TBI.

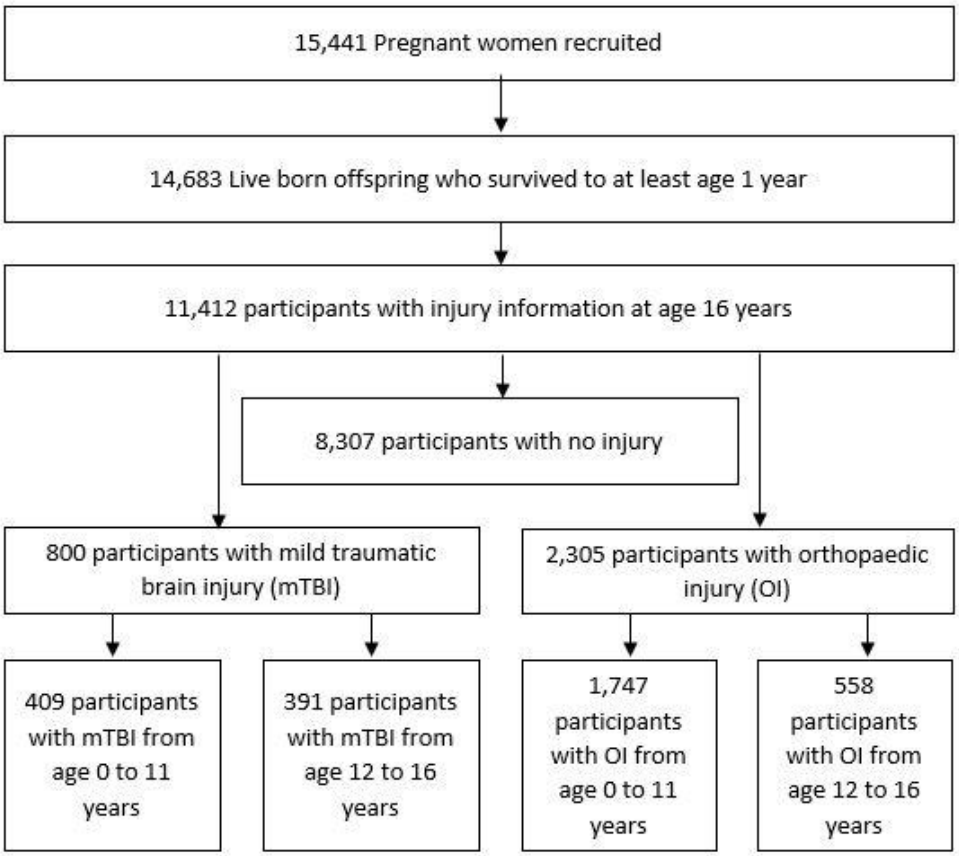

Figure 3-1 Flow chart of the final sample

Table 3-1 Descriptive statistics for covariates; Injuries from birth to age 16 years

	No Injury (n=8,307)	**TBI** (n=800)	**OI** (n=2,305)	p value*
	N (%)	N (%)	N (%)	
Male	4,109 (49.5)	457 (57.1)	1,283 (55.7)	<0.001
Social Class IV – V [a]	3052 (42.9)	273 (37.7)	786 (39.5)	0.001
Rented subsidised housing	967 (12.5)	60 (8.0)	181 (8.5)	<0.001
Mother completed secondary school	4,826 (63.4)	434 (57.7)	1,236 (58.9)	<0.001
Maternal daily smoking	2,246 (28.6)	212 (27.6)	576 (26.7)	0.186
Maternal daily alcohol use	989 (12.6)	110 (14.3)	309 (14.3)	0.067
Three or more early life events [b]	4,107 (52.9)	470 (61.6)	1,220 (57.1)	<0.001
	M (SD)	M (SD)	M (SD)	
Maternal age at birth (years)	28.42 (4.76)	28.92 (4.76)	28.72 (4.74)	0.001
Bonding at 8 months [c]	28.25 (3.68)	28.08 (3.55)	28.20 (3.59)	0.512
Positive parenting experience at 21 months [d]	5.99 (1.51)	6.01 (1.53)	6.00 (1.55)	0.934
Negative parenting experience at 21 months [d]	20.80 (2.74)	20.63 (2.84)	20.77 (2.73)	0.281

TBI: traumatic brain injury; OI: orthopaedic injury; ***** p values calculated using chi square or analysis of variance; [a] highest social class of either parent is skilled non-manual or lower occupation based on the Registrar General's classification of occupations; [b] parent-reported questionnaire relating to upsetting events in the child's life completed when offspring was 6, 30, 42 and 81 months old; [c] parent-report questionnaire completed when offspring was 8 months old; [d] positive and negative parenting experiences based on parent-completed questionnaire when offspring was 21 months old.

3.3.2 Associations with Alcohol, Tobacco and Cannabis Use

Individuals with TBI were at increased odds of hazardous use of alcohol (unadjusted OR 1.51, 95% CI 1.21 to 1.90), problematic use of tobacco (unadjusted OR 1.47, 95% CI 1.12 to 1.94) and problematic use of cannabis (unadjusted OR 1.54, 95% CI 1.22 to 1.94). These associations were robust to adjustment for pre-birth and childhood confounders. Mutual adjustment for the other substance use variables weakened the associations of TBI with alcohol use, and fully attenuated the association with tobacco and cannabis use. In the negative control analyses, OI was associated with cannabis use (unadjusted OR 1.22, 95% CI 1.04 to 1.43), but this was attenuated following adjustment for pre-birth and childhood confounders. There was no evidence for any associations between OI and alcohol or tobacco use, implying that the associations observed are specific to TBI. In the direct comparison to those with OI, participants with TBI were at increased odds of hazardous alcohol use only (unadjusted OR 1.34, 95% CI 1.05 to 1.72). These results are shown in Table 3.2. Excluding participants with both TBI and OI strengthened the association between TBI and alcohol use (unadjusted OR 1.48, 95% CI 1.10 to 1.99; adjusted OR 1.57, 95% CI 1.13 to 2.18); findings from this analysis can be seen in appendices 3.4 and 3.5. The findings from the complete case analyses did not differ substantially and can be seen in appendix 3.3.

Table 3-2 Associations between traumatic brain injury and orthopaedic injuries from birth to age 16 years and substance use at age 17 years

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 3694	n = 3263	n = 2884	n = 2074
TBI vs no Injury	1.51 (1.21 to 1.90)	1.46 (1.15 to 1.85)	1.56 (1.21 to 2.01)	1.31 (0.94 to 1.82)
OI vs no Injury	1.13 (0.97 to 1.31)	1.06 (0.90 to 1.25)	1.06 (0.89 to 1.27)	0.77 (0.61 to 0.98)
TBI vs OI	1.34 (1.05 to 1.72)	1.37 (1.06 to 1.79)	1.47 (1.11 to 1.94)	1.69 (1.17 to 2.45)
Omnibus p	0.045	0.265	0.251	0.080
	Tobacco [b]**			
n	n = 3099	n = 2741	n = 2420	n = 2074
TBI vs no Injury	1.47 (1.12 to 1.94)	1.51 (1.12 to 2.03)	1.46 (1.06 to 2.01)	1.09 (0.74 to 1.62)
OI vs no Injury	1.16 (0.96 to 1.42)	1.20 (0.97 to 1.49)	1.22 (0.97 to 1.54)	1.15 (0.86 to 1.55)
TBI vs OI	1.26 (0.93 to 1.72)	1.26 (0.91 to 1.74)	1.19 (0.84 to 1.70)	0.95 (0.61 to 1.47)
Omnibus p	0.060	0.044	0.050	0.331
	Cannabis [c]**			
n	n = 3979	n = 3505	n = 3090	n = 2074
TBI vs no Injury	1.54 (1.22 to 1.94)	1.36 (1.06 to 1.75)	1.39 (1.07 to 1.80)	1.23 (0.87 to 1.74)
OI vs no Injury	1.22 (1.04 to 1.43)	1.15 (0.97 to 1.37)	1.15 (0.96 to 1.39)	1.02 (0.79 to 1.33)
TBI vs OI	1.26 (0.98 to 1.62)	1.18 (0.90 to 1.55)	1.20 (0.90 to 1.60)	1.20 (0.82 to 1.77)
Omnibus p	0.004	0.054	0.071	0.718

Sample size reduces per adjustment as the participants who are missing covariate data get excluded TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

3.3.3 Associations with Offences and Trouble with the Police

Individuals with TBI were more likely to have committed at least one offence (unadjusted OR 1.72, 95% CI 1.32 to 2.23) and to have been in trouble with the police (unadjusted OR 1.62, 95% CI 1.21 to 2.17). The association with committing at least one offence was robust to adjustment for pre-birth and childhood confounders, while the association with being in trouble with the police was attenuated. Further adjustment for substance use variables substantially weakened the associations between TBI and offences and TBI and trouble with the police. In the negative control analyses, OI was associated with criminal behaviours (offences: unadjusted OR 1.48, 95% CI 1.23 to 1.77; trouble with the police: unadjusted OR 1.42, 95% CI 1.15 to 1.74), but while the association with offences was robust to adjustment for pre-birth and childhood confounders and substance use, the association with trouble with the police was attenuated substantially following adjustment. There was no clear evidence for increased odds of either offending or being in trouble with the police in the direct comparison between TBI and OI. These results are shown in Table 3.3. The findings from the complete case and additional sensitivity analyses did not differ substantially and can be seen in appendices 3.6, 3.7 and 3.8.

Table 3-3 Associations between traumatic brain injury and orthopaedic injuries from birth to age 16 years and criminal behaviours at age 17 years

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 3846	n = 3396	n = 2990	n = 2115
TBI vs no Injury	1.72 (1.32 to 2.23)	1.56 (1.17 to 2.07)	1.67 (1.24 to 2.24)	1.29 (0.09 to 1.88)
OI vs no Injury	1.48 (1.23 to 1.77)	1.35 (1.11 to 1.65)	1.41 (1.14 to 1.74)	1.67 (1.27 to 2.19)
TBI vs OI	1.16 (0.87 to 1.54)	1.15 (0.85 to 1.56)	1.18 (0.86 to 1.63)	0.77 (0.52 to 1.16)
Omnibus p	<0.001	0.001	0.001	<0.001
	Trouble with the Police [b]**			
n	n = 3782	n = 3340	n = 2947	n = 2077
TBI vs no Injury	1.62 (1.21 to 2.17)	1.33 (0.96 to 1.84)	1.44 (1.03 to 2.01)	1.17 (0.77 to 1.77)
OI vs no Injury	1.42 (1.15 to 1.74)	1.21 (0.97 to 1.52)	1.23 (0.96 to 1.56)	1.03 (0.75 to 1.42)
TBI vs OI	1.14 (0.83 to 1.57)	1.09 (0.77 to 1.55)	1.17 (0.81 to 1.69)	1.14 (0.71 to 1.81)
Omnibus p	<0.001	0.064	0.062	0.765

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

3.3.4 Associations with Conduct Problems and Peer Problems

Participants with TBI were at increased risk of having conduct problems (unadjusted OR 1.58, 95% CI 1.11 to 2.25), and this association was slightly strengthened following adjustment for pre-birth and childhood confounders. There was no evidence for an association between TBI status and peer problems. In the negative control analyses, there was no evidence for an association between OI status and conduct or peer problems. There was no clear evidence for increased odds of either conduct or peer problems in the direct comparison between TBI and OI. These results are shown in Table 3.4. The findings from the complete case and additional sensitivity analyses did not differ substantially and can be seen in appendices 3.9, 3.10 and 3.11.

Table 3-4 Associations between traumatic brain injury and orthopaedic injuries from birth to age 16 years and psychiatric symptoms based on the Strengths and Difficulties Questionnaire (SDQ) at age 17 years

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 5634	n = 4997	n = 4493
TBI vs no Injury	1.58 (1.11 to 2.25)	1.78 (1.22 to 2.59)	1.62 (1.08 to 2.41)
OI vs no Injury	1.15 (0.87 to 1.50)	1.12 (0.83 to 1.52)	1.07 (0.78 to 1.47)
TBI vs OI	1.38 (0.93 to 2.05)	1.58 (1.03 to 2.42)	1.51 (0.96 to 2.37)
Omnibus p	0.181	0.242	0.445
	Peer Problems [b]*		
n	n = 5626	n = 4987	n = 4483
TBI vs no Injury	1.11 (0.79 to 1.55)	0.99 (0.68 to 1.42)	0.85 (0.57 to 1.26)
OI vs no Injury	0.96 (0.76 to 1.22)	0.81 (0.62 to 1.06)	0.79 (0.60 to 1.05)
TBI vs OI	1.15 (0.79 to 1.67)	1.21 (0.80 to 1.83)	1.07 (0.68 to 1.67)
Omnibus p	0.852	0.138	0.090

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

3.3.5 Effects of Age at Injury: Childhood and Adolescent Injuries

Both childhood (between birth and age 11 years) and adolescent (between age 12 and 16 years) TBI were associated with problematic cannabis use at age 17 years in the unadjusted models (childhood: unadjusted OR 1.61, 95% CI 1.14 to 2.28; adjusted OR 1.45, 95% CI 0.98 to 2.15; adolescent: unadjusted OR 1.49, 95% 1.11 to 1.99; adjusted OR 1.36, 95% CI 0.98 to 1.88). Adolescent TBI was also associated with increased hazardous use of alcohol at age 17 years (unadjusted OR 1.71, 95% CI 1.28 to 2.27; adjusted OR 1.72, 95% CI 1.25 to 2.37) and problematic use of tobacco at age 17 years (unadjusted OR 1.56, 95% 1.11 to 2.19; adjusted OR 1.71, 95% CI 1.15 to 2.52). In the negative control analyses, adolescent OI was associated with problematic use of tobacco (unadjusted OR 1.50, 95% CI 1.13 to 2.00; adjusted OR 1.76, 95% CI 1.25 to 2.48). There was no evidence of an association between OI status and any of the other substance use measures. Relative to adolescent OI, adolescent TBI was associated with increased odds of alcohol use only (unadjusted OR 1.61, 95% CI 1.13 to 2.31; adjusted OR 1.76, 95% CI 1.17 to 2.63).

The adolescent TBI group were more likely to have committed at least one offence at age 17 years (unadjusted OR 2.05, 95% CI 1.50 to 2.80; adjusted OR 1.99, 95% CI 1.40 to 2.82) and to have been in trouble with the police at age 17 years (unadjusted OR 1.74, 95% CI 1.22 to 2.48; adjusted OR 1.51, 95% CI 1.00 to 2.29). In negative control analyses, adolescent OI was associated with having committed at least one offence (adolescent: unadjusted OR 1.89, 95% CI 1.44 to 2.45; adjusted OR 1.53, 95% CI 1.11 to 2.11).

Childhood TBI was associated with increased conduct problems on the SDQ at age 17 years (unadjusted OR 2.20, 95% CI 1.37 to 3.53; adjusted OR

1.90, 95% CI 1.11 to 3.26). As DAWBA information was available at age 15 years, odds ratios were also calculated for the association between childhood TBI and externalising disorders from this scale. DAWBA externalising symptoms are a combination of ODD, CD and ADHD symptoms. The results of the DAWBA analysis can be seen in appendix 3.18. Participants with childhood TBI were more likely to have externalising symptoms (unadjusted OR 2.25, 95% CI 1.32 to 3.81; adjusted OR 1.83, 95% CI 0.98 to 3.41). Analyses of the three separate disorders revealed a strong effect size of TBI on ADHD (adjusted OR 3.15, 95% CI 1.07 to 9.28). Relative to childhood OI, childhood TBI was associated with increased odds of conduct problems (unadjusted OR 2.10, 95% CI 1.23 to 3.57; adjusted OR 1.98, 95% CI 1.08 to 3.65) and externalising symptoms (unadjusted OR 2.65, 95% CI 1.43 to 4.91; adjusted 2.11, 95% CI 1.03 to 4.32). The full sample and complete case analyses for childhood and adolescent injuries are provided in appendix 3.12 to appendix 3.39.

3.4 DISCUSSION

I used data from a longitudinal birth cohort to explore the association between sustaining a mTBI before age 16 years and subsequent substance use, criminal behaviour and psychiatric symptoms. There are three main findings. First, relative to having no injury, sustaining a mTBI between birth and age 16 was associated with problematic alcohol, tobacco and cannabis use, a higher likelihood of committing an offence and a higher likelihood of having conduct problems at age 17 years. Second, in negative control analyses, there was evidence that sustaining a mTBI was associated with hazardous alcohol use relative to sustaining an OI – adding evidence for a possible causal association

between TBI and later alcohol misuse – while both mTBI and OI were associated with committing offences. Third, additional analyses suggest that age at injury may be important for certain outcomes; participants with a mTBI between birth and age 11 years had higher odds of psychiatric symptoms and problematic cannabis use at age 17 years, while participants who incurred a mTBI between age 12 and 16 years had higher odds of problematic substance use and criminal behaviours at age 17 years.

The first main finding lends support to results from other birth cohort studies; although the strength of evidence for associations found in my study are somewhat weaker than those in other birth cohorts. This could reflect my use of self-report rather than medical records whereby non-TBI events may be recalled as TBI diluting the true exposure. I found that 7% of the cohort had experienced a TBI by age 16 years, this lies between the rate of 3.8% in the Northern Finland cohort and 31.6% in the CHDS (Corrigan, Selassie, & Orman, 2010). MTBI was associated with 39% to 67% increased risk of six of the seven outcomes, which is comparable to the 18% to 52% increased risk of poor adult outcomes reported by Sariaslan and colleagues (Sariaslan et al., 2016). The increased odds of higher levels of alcohol consumption amongst the TBI group here is in keeping with the more frequent intoxication reported by 14 year olds with a TBI in The Northern Finland Birth Cohort (Winqvist et al., 2007). In the CHDS, a TBI requiring hospitalisation was associated with increased odds of externalising disorders and substance abuse at age 14 to 16 years (McKinlay et al., 2009) and with increased odds of alcohol and drug dependence, and criminal behaviour at age 16 to 25 years (McKinlay et al., 2014). In the current investigation, the association between TBI and criminal offences did not remain once substance use was added

as a covariate. McKinlay and colleagues reported a similar association for those injured before age 5 years; however, for those injured from age 6 to 15 years a strong association remained for arrests and property offences, but not violent offences. They concluded that a certain threshold of TBI may be required for these effects to be seen (McKinlay et al., 2014). However, in this study it was not possible to index the severity of the TBI.

Second, I included a negative control exposure group to increase the confidence that the associations seen between TBI and risk behaviour may be causal, where several previous studies have only used an uninjured control group (Kennedy, Cohen, & Munafò, 2017). I found that participants who had sustained an OI were not at increased risk of problematic substance use or conduct problems compared to the no injury group, providing further support to previous literature. Interestingly, in a direct comparison between the two injury groups, the TBI group were only found to have a higher likelihood of hazardous alcohol use. This finding has implications for the treatment and management of youth post-TBI as alcohol use has previously been linked with recurrent TBI (Winqvist et al., 2008) and poorer recovery from TBI (Corrigan, 1995). The lack of evidence for an association between TBI and the other risk outcomes when directly compared with OI highlights the importance of exercising caution when drawing conclusions about mTBI from research that does not take other injuries into account. The association between OI and committing offences was an unexpected finding; one plausible explanation is that there may be common risk factors for both committing crimes and for being involved in accidents that result in physical injury. For example, sensation-seeking has previously been linked with both criminality and spinal cord injuries in a case-control study of 140 male spinal cord

injury patients and 140 matched controls (Mawson et al., 1996). Although both TBI and OI were associated with committing offences when compared to the no injury control group, only those with a TBI were more likely to have been in trouble with the police. Previously it has been suggested that having a TBI may be a risk factor for criminal behaviour and it may place an individual at a disadvantage during legal proceedings (Williams, McAuliffe, Cohen, Parsonage, & Ramsbotham, 2015). My finding raises the possibility that having a TBI may also be a factor in the initial transition into the legal system. Future studies in prison populations should measure the incidence rate of OI in addition to TBI in order to further explore this relationship.

Third, there were some differences in risk of outcomes for childhood and adolescent TBI. Childhood TBI (aged 0 – 11 years) was associated with conduct problems, while adolescent TBI (aged 12 – 16 years) was associated with increased likelihood of problematic alcohol and tobacco use, as well as criminality. Adolescent OI was associated with problematic tobacco use and committing offences, further highlighting a possible role for common risk factors mentioned above. TBI in both age groups showed weak association with cannabis use. Between these age ranges there was a change from parent-reported to self-reported TBI, however I feel that this change is unlikely to have impacted the findings as it is more appropriate for the offspring to report their own injuries once they have entered secondary education. There may be some differences in severity of the injuries reported from childhood to adolescence – elsewhere the injuries occurring after 15 years were more severe (Corrigan et al., 2010) – it would be interesting to assess this possibility in future research.

3.4.1 Strengths and Limitations

The prospective birth cohort design is a major strength of this study. Each injury was reported in close proximity to the time it happened, minimising the issue of recall bias. The longitudinal nature of the study allows for causal inference based on the temporal relationship between exposure and outcome. Additionally, the lack of statistical support and weak associations between my negative control group and the main outcomes, with the exception of committing offences, adds to the strength of evidence for a causal association suggested by previous research. On the other hand, the findings from the direct comparison between TBI and OI showing that TBI was only associated with hazardous alcohol use highlights the importance of exercising caution when interpreting findings on mTBI without inclusion of a negative control group. However, the study is not without limitations. In particular, I was unable to obtain any index of severity based on the TBI measure, meaning that some nuances in effects based on severity may have been missed. For example, increased alcohol use has previously been related to mild but not moderate-to-severe TBI (Winqvist et al., 2007). Nonetheless the items used to identify TBI are similar to existing research, skull fractures based on ICD codes have been used to classify mTBI elsewhere (Winqvist et al., 2007) and self-report questions asking about loss of consciousness have also been utilised (Moore, Indig, & Haysom, 2014; Perron & Howard, 2008; Williams, Cordan, Mewse, Tonks, & Burgess, 2010).

3.4.2 Chapter summary

Evidence from cross sectional work suggests that there is a relationship between mTBI and risk behaviour in youth (Ilie et al., 2014, 2015, 2016; Max et al., 1998; Tonks et al., 2011); however, there is a paucity of high quality longitudinal research investigating this association (Kennedy et al., 2017). I have attempted to further explore a potential causal link by using data from a representative birth cohort and including a non-brain related injury group as a negative exposure control. Overall, I found that participants who sustained a mTBI before age 16 years were more likely than those with no injury or with a history of OI to use alcohol to problematic levels at age 17 years. Additionally, sustaining either a mTBI or OI before age 16 years increased the likelihood of an individual committing offences at age 17 years. This chapter adds evidence for a possible causal effect of mTBI in youth on later hazardous alcohol use and highlights the importance of including an extra injury group in mTBI research. However, the underlying mechanism related to this association remains unclear. In the next chapter, I will explore the neuropathology of mTBI in a subsample of the same cohort. Using MRI techniques that assess brain microstructure, I aim to elucidate differences between the three participant groups in brain microstructure and explore neural substrates related to alcohol use and mTBI.

Chapter 4 Magnetic Resonance Imaging (MRI) based measures associated with mTBI

4.1 BACKGROUND

Chapters 2 and 3 presented evidence to suggest that mTBI may be associated with behavioural outcomes such as substance use, criminal behaviours and psychiatric issues in the months and years following injury. It is possible that brain changes following mTBI may contribute to these associations. For example, damage to regions of the brain implicated in executive function could result in impaired executive control leading to increases in risk taking behaviour. As introduced in Chapter 1, section 1.3.3, neuroimaging methods that assess brain microstructure have been sensitive to mTBI.

In this chapter, I report evidence from a study using four MRI-based measures of grey and white matter to explore the association of mTBI with brain microstructure. A secondary aim was to further investigate the relationship between alcohol use and mTBI as there is evidence to suggest this relationship may be bidirectional. For example, participants in the Northern Finland Birth Cohort who reported frequent consumption of alcohol or occasionally being drunk at age 14 years had an increased risk of TBI up to age 35 years (Winqvist, Jokelainen, Luukinen, & Hillbom, 2006). I aimed to explore the influence of alcohol use on brain microstructure, and to further explore how differences observed in the mTBI group may be influenced by alcohol use.

Data for the current chapter were drawn from a subsample of male participants from ALSPAC aged between age 19 and 21 years. A negative control

exposure group with a history of orthopaedic injury was included in the analyses. The four MRI-based measures of the whole cortex and four lobes explored were T1 relaxation time, T2 relaxation time, myelin water fraction (MWF) and magnetisation transfer ratio (MTR). These measures have been introduced in more detail in Chapter 1 section 1.3.1.3. Briefly, T1 and T2 relaxation time are measured in milliseconds; for both measures, a longer time indicates a greater presence of water/less brain microstructure obstructing the movement of water molecules. MWF is a measure of water in the myelin bilayers and MTR is a measure of myelin-bound water.

4.2 METHODS

4.2.1 Participants

The sample was drawn from the Avon Longitudinal Study of Parents and Children (ALSPAC); this is an ongoing birth cohort study based in the South West region of England. Pregnant women residing in the area with an expected delivery date between 1 April 1991 and 31 December 1992 were invited to participate, resulting in 14,451 pregnancies recruited. Details of all available data can be found on the study website through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

A subset of 507 male participants underwent magnetic resonance imaging (MRI) as part of a National Institutes of Health-funded project investigating associations between axons, testosterone and mental health. Participants were aged between 18 and 21 years (mean 19.63, SD 1.84 months) at the time of

scanning and those with injury data between age 14 and 16 years were included in the present study.

4.2.2 Questionnaire Measures

4.2.2.1 Injury groups

Participants completed two self-report questionnaires related to injury history. At age 15 years participants reported on fractures incurred since their 12th birthday, including skull fractures. At age 16 years participants reported on a head injury since their 14th birthday or fractures in the last 6 months and provided details of the head injury in a free text box. Participants in the mTBI group were identified based on a positive response to the item "have you had a head injury resulting in a loss of consciousness" or specification of a skull fracture in either fracture questionnaire. Participants in the orthopaedic injury group were identified by a positive response to breaking a bone on the upper limb, lower limb or other bone (excluding skull fracture). Those who reported a fracture before age 14 years were excluded from the analysis. Similar postal questionnaires had been completed by the parents asking about injuries their offspring had incurred until age 11 years; these responses were also used to exclude any participants who had sustained an injury before age 14 years.

4.2.2.2 Alcohol use

Data on alcohol use was based on a self-report questionnaire at age 13 years. Items used to assess alcohol use were that the participant had "tried alcohol without parents' permission" and had "ever had a whole drink of alcohol".

4.2.3 MRI Acquisition

Images were acquired on a GE 3T magnet, using an 8-channel, receiver-only head coil. Magnetisation Transfer Ratio was estimated from images obtained using a 3D spoiled gradient recalled (SPGR) sequence in the sagittal plane with the following parameters: voxel size = 1.9 × 1.9 × 1.9mm^3; field of view = 240 × 240 mm^2; matrix size =128 × 128; slice thickness = 1.9 mm; number of slices = 100; TR/TE = 26.7 ms/1.8 ms; flip angle = 5°, parallel imaging acceleration factor (ASSET) = 2 (acquisition time of 4:27 min), MT pulse frequency offset = 2 kHZ and effective flip angle = 450°. For T1, T2 and Myelin Water Fraction, a multicomponent equilibrium single-pulse observation of T1 and T2 (mcDESPOT) using a 3D fast spoiled gradient recalled (FSPGR). For each participant a total of 25 sagittal images were acquired with the following parameters: field of view = 220×220×163 mm and an acquisition matrix of 128×128×88 (1.72×1.72 mm in-plane resolution). These 25 images included 8 T1-weighted spoiled gradient recalled echo images (SPGR: TE = 2.112 ms, TR = 4.7 ms, flip angles = 3°, 4°, 5°, 6°, 7°, 9°, 13° and 18°); 2 inversion-prepared SPGR images (IRSPGR: TE = 2.112 ms, TR = 4.7 ms, IR = 450 ms, flip angle = 5°); and 15 T1/T2 weighted steady-state free precession (SSFP) images (TE = 1.6 ms TR = 3.2 ms, flip angles of 10.59°, 14.12°, 18.53°, 23.82°, 29.12°, 35.29°, 45°, 60° and phase-cycling angles of 0° and 180°).

4.2.4 MRI-based Measures

There were four derived MRI parameters; T1 relaxation time, T2 relaxation time, myelin water fraction (MWF) and magnetisation transfer ratio (MTR). Average values for each of these parameters were obtained separately for

the grey and white matter of: a) the whole cerebrum (excluding subcortical grey matter), i.e. global measures, and b) each of the four lobes (frontal, parietal, temporal and occipital lobe), i.e. lobar measures. First, tissue segmentation was performed on all voxels, determining whether a voxel contained grey matter, white matter or cerebral spinal fluid. Next, native parametric images were registered to native T1w images using ANTs intermodality-intrasubject rigid (MTR) and rigid & nonlinear (mcDESPOT) transforms. T1w images were then nonlinearly registered to the ICBM152 1 mm template. Masks of the four lobes in the right and left hemispheres were then projected from the ICBM152 space into native parametric images (MTR, mcDESPOT) using the inverse of the above intermodality and native-to-template transforms. Then, the intersection of each lobar mask and the tissue segmentation of grey and white matter was used to extract the mean value in all parametric maps. The whole cortex value for each parameter was based on the average of all voxels identified in the lobar masks as either grey matter or white matter. A single value was obtained for each of the four lobes by averaging the right hemisphere and left hemisphere grey or white matter value for that lobe. Grey and white matter were analysed separately.

4.2.5 Statistical analysis

Associations between each of four the MRI measures and: 1) injury history at age 14 to16 years; 2) alcohol use at age 13 years; and 3) the interaction effect of alcohol use and brain injury were planned for investigation. Linear regression analyses were conducted to explore the associations with the whole cortex MRI measures. Repeated measures mixed models with unstructured within-subject covariances were used for the lobar data, as data from the lobes were highly correlated. Post-hoc simple effects of lobe in each group were

conducted to explore associations with MRI measures in each lobe. Grey and white matter were analysed separately. All analyses were conducted using Stata version 13 (StataCorp LP, Texas).

4.3 RESULTS

4.3.1 Participants

Descriptive statistics for the participants can be seen in Table 4.1 and a flow chart of the final sample can be seen in Figure 4.1. In total there were 391 male participants who underwent MRI and had injury information. There were 48 participants with a reported mTBI and 65 participants with a reported OI between age 14 and 16 years with no injury before; 278 participants reported neither injury. There were 9 individuals who sustained a mTBI and an OI between age 14 and 16 years. Participants who sustained a mTBI provided additional information in a free text format, based on this information the majority of mTBIs were sport-related. The three groups did not differ in terms of any of the demographic characteristics assessed.

Table 4-1 Descriptive Statistics for the scanned subsample

	No Injury (n=278)	**TBI** (n=48)	**OI** (n=65)	p value*
	N (%)	N (%)	N (%)	
Social class IV – V [a]	67 (26.9)	12 (28.6)	17 (28.3)	0.958
Rented subsidised housing	11 (4.2)	0 (0)	1 (1.6)	0.271
Mother completed secondary school	102 (39.4)	18 (40.9)	28 (46.7)	0.585
Maternal daily smoking	43 (16.7)	4 (9.1)	13 (21.3)	0.249
Maternal daily alcohol use	31 (12.0)	11 (25)	9 (14.8)	0.071
Three or more early life events [b]	133 (51.2)	29 (65.9)	36 (58.1)	0.540
Two or more antisocial activities [c]	20 (8.1)	1 (2.2)	4 (6.5)	0.581
Drank alcohol	58 (21.3)	10 (21.3)	17 (26.2)	0.693
Cause of TBI				
Sport	-	33 (70.2)	-	-
Fall	-	5 (10.6)	-	-
Assault	-	3 (6.4)	-	-
Alcohol-related incident	-	2 (4.3)	-	-
Motor vehicle accident	-	1 (2.1)	-	-
Skull fracture	-	1 (2.1)	-	-
Unknown	-	2 (4.3)	-	-
	M (SD)	M (SD)	M (SD)	
Maternal age at birth (years)	30.06 (0.26)	30.04 (0.66)	29.96 (0.57)	0.988
Bonding at 8 months [d]	27.7 (3.6)	28.0 (3.3)	28.2 (3.4)	0.652
Positive parenting experience at 21 months [e]	6.0 (1.4)	6.0 (1.3)	5.81 (1.0)	0.465
Negative parenting experience at 21 months [e]	20.7 (2.6)	20.2 (2.9)	21.2 (2.8)	0.225
Total IQ score [f]	110.6 (1.07)	111.8 (2.63)	112.2 (2.15)	0.784

TBI: traumatic brain injury; OI: orthopaedic injury; ***** p values calculated using chi square or analysis of variance; [a] highest social class of either parent is skilled non-manual or lower occupation based on the Registrar General's classification of occupations; [b] parent-reported questionnaire relating to upsetting events in the child's life completed when offspring was 6, 30, 42 and 81 months old; [c] antisocial activities based on self-report at age 8 years [d] parent-report questionnaire completed when offspring was 8 months old; [e] positive and negative parenting experiences based on parent-completed questionnaire when offspring was 21 months old; [f] IQ based on the WISC at age 8 years

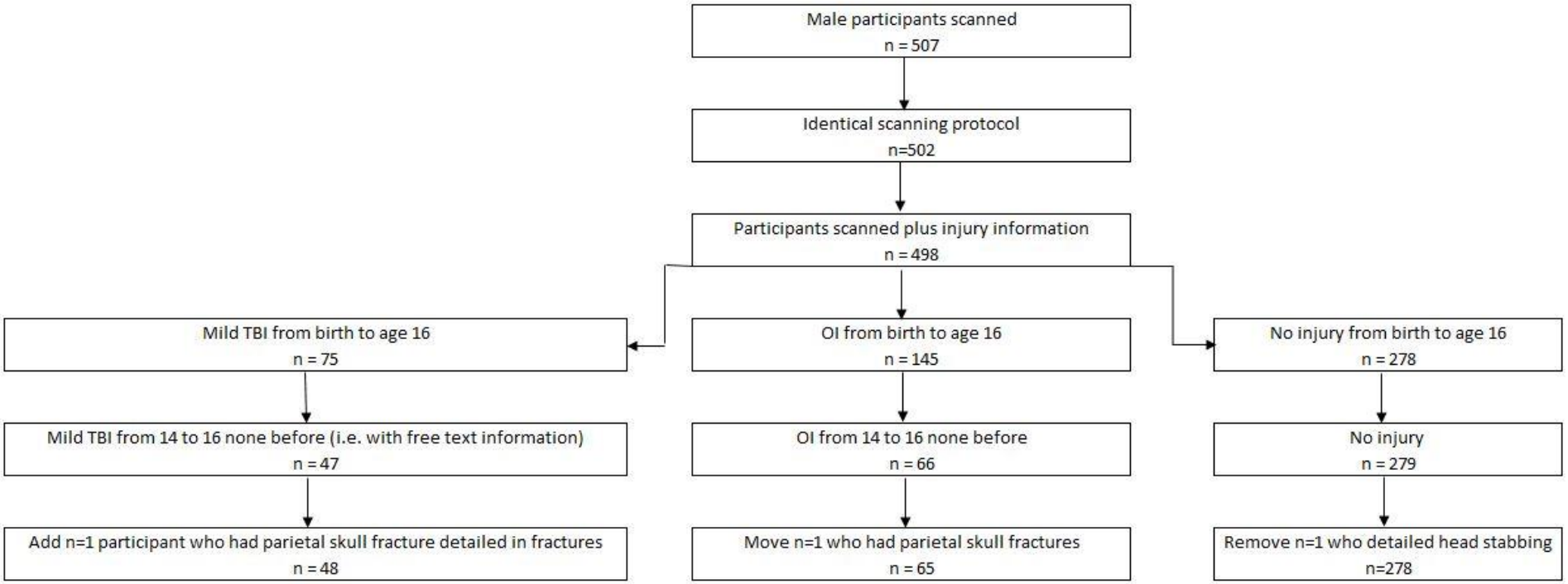

Figure 4-1 Flow chart of the final sample

4.3.2 Injury status

4.3.2.1 Global Measures

There was no clear evidence for an association between history of mTBI in MRI-based measures of global white matter in comparison to participants with no injury. Unexpectedly however, participants with OI had shorter T1 and T2 relaxation time and higher MWF and MTR in global measures of white matter than participants with no injury; and also, shorter T1 relaxation time and higher MWF than participants with mTBI, see Table 4.2. There was no evidence for any association between injury status and grey matter global measures, see Table 4.3.

4.3.2.2 Lobar Measures

There was no evidence for an association between history of mTBI in MRI-based measures of lobar white matter in comparison to participants with no injury. However, participants with OI had shorter T1 relaxation time in all four lobar measures of white matter; shorter T2 relaxation time in frontal and parietal lobes; and higher MWF in all four lobar measures of white matter when compared to those with no injury. Additionally, relative to those with mTBI, participants with OI had shorter T1 relaxation time in frontal and temporal lobe white matter; higher MWF in frontal and parietal lobe white matter; and higher MTR in parietal lobe white matter, see Table 4.4. There was no evidence for any association between injury status and grey matter lobar measures, see Table 4.5.

Table 4-2 White matter global measures

	TBI vs No Injury			OI vs No Injury			TBI vs OI		
	Coef.	95% CI	p value	Coef.	95% CI	p value	Coef.	95% CI	p value
T1	0.802	(-15.040 to 16.643)	0.921	-18.853	(-32.831 to -4.875)	0.008	19.655	(0.396 to 38.914)	0.045
T2	-0.150	(-1.020 to 0.720)	0.735	-0.768	(-1.535 to 0.001)	0.050	0.618	(-0.440 to 1.675)	0.251
MWF	<0.001	(-0.004 to 0.004)	0.889	0.005	(0.001 to 0.009)	0.008	-0.005	(-0.010 to 0.001)	0.040
MTR	-0.002	(-0.006 to 0.001)	0.243	0.002	(-0.001 to 0.005)	0.206	-0.004	(-0.009 to 0.001)	0.061

TBI: traumatic brain injury from 14 to 16 years; OI: orthopaedic injury from 14 to 16 years
T1: T1 relaxation time; T2: T2 relaxation time; MWF: myelin water fraction; MTR: magnetisation transfer ratio
Models adjusted for pre-birth confounders (mother's age at birth, mother's education at birth, social class) and for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Note in fully adjusted model: No Injury n = 227; TBI n = 39; OI n = 51

Table 4-3 Grey matter global measures

	TBI vs No Injury			OI vs No Injury			TBI vs OI		
	Coef.	95% CI	p value	Coef.	95% CI	p value	Coef.	95% CI	p value
T1	-8.625	(-35.873 to 18.623)	0.534	-18.907	(-42.950 to 5.136)	0.123	10.282	(-22.844 to 43.408)	0.542
T2	-0.360	(-2.804 to 2.084)	0.772	-1.731	(-3.887 to 0.425)	0.115	1.371	(-1.599 to 4.341)	0.364
MWF	-0.001	(-0.005 to 0.003)	0.753	0.002	(-0.001 to 0.006)	0.238	-0.003	(-0.008 to 0.002)	0.265
MTR	-0.001	(-0.005 to 0.002)	0.492	0.001	(-0.002 to 0.004)	0.402	-0.002	(-0.007 to 0.002)	0.241

TBI: traumatic brain injury from 14 to 16 years; OI: orthopaedic injury from 14 to 16 years
T1: T1 relaxation time; T2: T2 relaxation time; MWF: myelin water fraction; MTR: magnetisation transfer ratio
Models adjusted for pre-birth confounders (mother's age at birth, mother's education at birth, social class) and for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Note in fully adjusted model: No Injury n = 227; TBI n = 39; OI n = 51

Table 4-4 White matter lobar measures

	TBI vs No Injury			OI vs No Injury			TBI vs OI		
	Coef.	95% CI	p value	Coef.	95% CI	p value	Coef.	95% CI	p value
T1									
frontal	1.429	(-14.447 to 17.306)	0.860	-19.790	(-33.804 to -5.776)	0.006	21.220	(1.913 to 40.526)	0.031
parietal	0.702	(-15.191 to 16.594)	0.931	-16.949	(-30.977 to -2.921)	0.018	17.651	(-1.675 to 36.977)	0.073
occipital	-3.866	(-22.459 to 14.728)	0.684	-21.670	(-38.117 to -5.223)	0.010	17.804	(-4.843 to 40.451)	0.123
temporal	3.044	(-13.410 to 19.497)	0.717	-16.677	(-31.208 to -2.146)	0.024	19.721	(-0.295 to 39.738)	0.053
T2									
frontal	0.117	(-0.756 to 0.991)	0.792	-0.767	(-1.538 to 0.005)	0.052	0.884	(-0.179 to 1.947)	0.103
parietal	-0.105	(-0.971 to 0.762)	0.813	-0.748	(-1.514 to 0.017)	0.055	0.644	(-0.410 to 1.698)	0.231
occipital	-0.650	(-1.807 to 0.508)	0.271	-0.854	(-1.880 to 0.171)	0.103	0.204	(-1.207 to 1.616)	0.777
temporal	-0.248	(-1.381 to 0.885)	0.668	-0.662	(-1.665 to 0.342)	0.196	0.413	(-0.967 to 1.794)	0.557
MWF									
frontal	-0.001	(-0.006 to 0.003)	0.519	0.005	(0.001 to 0.009)	0.007	-0.007	(-0.012 to -0.001)	0.013
parietal	-0.001	(-0.005 to 0.003)	0.710	0.004	(0.001 to 0.008)	0.017	-0.005	(-0.010 to <0.001)	0.041
occipital	0.003	(-0.003 to 0.008)	0.303	0.006	(0.001 to 0.011)	0.014	-0.003	(-0.010 to 0.003)	0.349
temporal	0.001	(-0.004 to 0.005)	0.796	0.005	(0.001 to 0.008)	0.014	-0.004	(-0.009 to 0.001)	0.115
MTR									
frontal	-0.002	(-0.006 to 0.002)	0.341	0.002	(-0.001 to 0.006)	0.197	-0.004	(-0.009 to 0.001)	0.085
parietal	-0.003	(-0.007 to 0.001)	0.193	0.002	(-0.001 to 0.006)	0.222	-0.005	(-0.010 to <0.001)	0.051
occipital	-0.003	(-0.007 to <0.001)	0.088	0.000	(-0.003 to 0.004)	0.778	-0.004	(-0.008 to 0.001)	0.109
temporal	-0.002	(-0.005 to 0.002)	0.275	0.001	(-0.002 to 0.004)	0.437	-0.003	(-0.007 to 0.001)	0.144

TBI: traumatic brain injury from 14 to 16 years; OI: orthopaedic injury from 14 to 16 years
T1: T1 relaxation time; T2: T2 relaxation time; MWF: myelin water fraction; MTR: magnetisation transfer ratio
Models adjusted for pre-birth confounders (mother's age at birth, mother's education at birth, social class) and for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Note in fully adjusted model: No Injury n = 227; TBI n = 39; OI n = 51

Table 4-5 Grey matter lobar measures

	TBI vs No Injury			OI vs No Injury			TBI vs OI		
	Coef.	95% CI	p value	Coef.	95% CI	p value	Coef.	95% CI	p value
T1									
frontal	-4.498	(-35.574 to 26.577)	0.777	-17.699	(-45.202 to 9.803)	0.207	-13.201	(24.665 to -51.067)	0.494
parietal	-11.793	(-39.760 to 16.174)	0.409	-15.383	(-40.105 to 9.338)	0.223	-3.591	(30.456 to -37.638)	0.836
occipital	-11.991	(-38.754 to 14.772)	0.380	-25.397	(-49.041 to -1.753)	0.035	-13.406	(19.161 to -45.973)	0.420
temporal	-3.936	(-29.897 to 22.024)	0.766	-18.680	(-41.605 to 4.244)	0.110	-14.744	(16.836 to -46.323)	0.360
T2									
frontal	-0.002	(-2.490 to 2.485)	0.999	-1.730	(-3.930 to 0.469)	0.123	-1.728	(1.300 to -4.756)	0.263
parietal	-0.350	(-3.543 to 2.844)	0.830	-1.733	(-4.563 to 1.098)	0.230	-1.383	(2.512 to -5.277)	0.486
occipital	-1.837	(-5.181 to 1.506)	0.281	-2.987	(-5.951 to -0.023)	0.048	-1.149	(2.929 to -5.227)	0.581
temporal	-0.551	(-2.790 to 1.689)	0.630	-1.160	(-3.137 to 0.818)	0.250	-0.609	(2.114 to -3.331)	0.661
MWF									
frontal	-0.003	(-0.008 to 0.002)	0.243	0.002	(-0.002 to 0.006)	0.309	0.005	(0.011 to -0.001)	0.090
parietal	0.000	(-0.004 to 0.004)	0.876	0.001	(-0.003 to 0.005)	0.567	0.001	(0.006 to -0.004)	0.587
occipital	0.002	(-0.002 to 0.006)	0.362	0.003	(-0.001 to 0.007)	0.178	0.001	(0.006 to -0.005)	0.818
temporal	0.001	(-0.003 to 0.004)	0.757	0.002	(-0.001 to 0.006)	0.185	0.002	(0.006 to -0.003)	0.478
MTR									
frontal	-0.001	(-0.004 to 0.003)	0.702	0.001	(-0.002 to 0.005)	0.418	0.002	(0.007 to -0.002)	0.366
parietal	-0.002	(-0.005 to 0.002)	0.418	0.002	(-0.001 to 0.006)	0.169	0.004	(0.009 to -0.001)	0.096
occipital	-0.002	(-0.005 to 0.002)	0.381	0.001	(-0.002 to 0.004)	0.646	0.002	(0.007 to -0.002)	0.293
temporal	-0.001	(-0.005 to 0.002)	0.423	0.000	(-0.003 to 0.003)	0.837	0.002	(0.006 to -0.002)	0.420

TBI: traumatic brain injury from 14 to 16 years; OI: orthopaedic injury from 14 to 16 years
T1: T1 relaxation time; T2: T2 relaxation time; MWF: myelin water fraction; MTR: magnetisation transfer ratio
Models adjusted for pre-birth confounders (mother's age at birth, mother's education at birth, social class) and for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Note in fully adjusted model: No Injury n = 227; TBI n = 39; OI n = 51

4.3.3 Alcohol use

4.3.3.1 Global Measures

There was weak evidence for an association between using alcohol at age 13 years and short T1 relaxation time in global grey matter (adjusted coefficient (coef.) -18.22, 95% CI -36.80 to 0.36). There was no evidence for an association between alcohol use at age 13 years and global measures of white matter.

4.3.3.2 Lobar Measures

There was moderate evidence that those who drank alcohol at age 13 had shorter T1 relaxation time in frontal and temporal measures of grey matter (coef. -23.94, 95% CI -45.56 to -2.33; and coef., -18.62, 95% CI -36.01 to -1.24 respectively). There was no evidence for an association between alcohol use at age 13 years and lobar measures of white matter.

4.3.4 Unexpected findings

The finding that OI was associated with MRI-based measures of global and lobar white matter microstructure was unexpected and warranted further investigation. I explored two possibilities – reverse causality and collider bias. Due to the nature of the injury findings, I did not pursue the effect of the interaction between alcohol use and mTBI.

4.3.4.1 Reverse Causality

Reverse causality occurs when the outcome causes the exposure, contrary to the expected causal direction; this may operate through a third variable, see Figure 4.2 (A). In this case, brain structure may have a causal association with an unknown variable that leads to OI. For this to be a plausible explanation, this

factor has to be specific to OI and not to the mTBI or no injury groups. I explored this in the full cohort (n = 9,254) using all of the demographic characteristics shown in Table 4.1 and only found weak evidence that individuals who reported one antisocial behaviour at age 8 years had increased risk of sustaining an OI between age 14 and 16 years relative to those with no injury (adjusted relative risk ratio 1.33, 95% CI 0.98 to 1.81).

4.3.4.2 Collider bias

Collider bias is a type of selection bias that occurs when two variables influence a third variable and this third variable is conditioned upon (Munafò, Tilling, Taylor, Evans, & Smith, 2018), see Figure 4.2 (B). In this case, I hypothesized that injury status and underlying brain structure may have independently influenced participation in this ALSPAC sub-study, inducing a spurious association between OI and MRI-measures, and potentially a null association between mTBI and MRI-based outcomes. I found that injury status predicted participation; in the full cohort those with mTBI and those with OI were at increased odds of participating (adjusted odds ratio (OR) 3.08, 95% CI 2.14 to 4.44; OR 3.45, 95% CI 2.49 to 4.78 respectively). In order to test whether underlying brain structure was related to participation, I attempted to use a genetic variant as a proxy for brain structure in the full cohort. However, I was unable to identify a genetic variant associated with the MRI modalities explored in the current study.

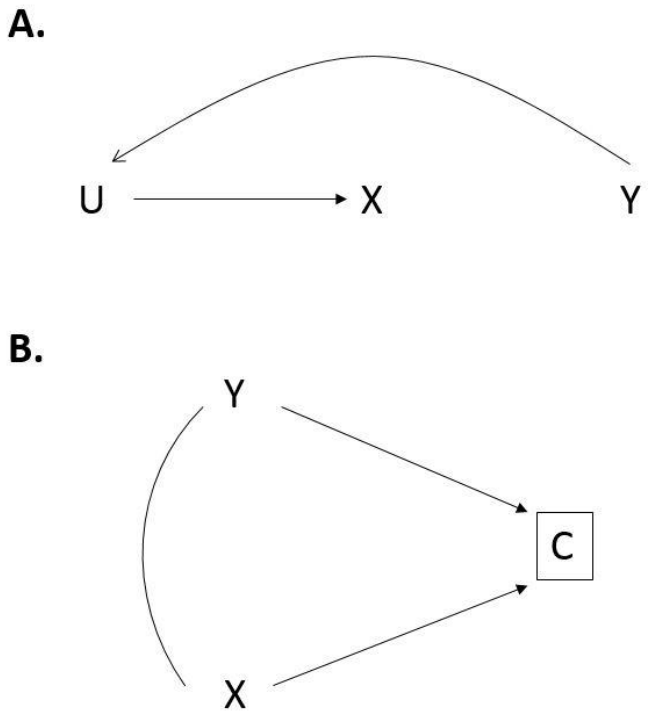

Figure 4-2 Causal diagrams representing reverse causality (A) and collider bias (B). Arrows represent the causal direction; X is the exposure and Y is the outcome. In the reverse causality diagram (A), U represents the unknown variable that is a result of the outcome Y and causes the exposure X. In the collider bias diagram (B), the boxed letter C represents the third variable that is conditioned upon; the line between X and Y represents the observed association induced by the presence of collider bias.

4.4 DISCUSSION

The aim of this chapter was to explore the association between mTBI and MRI-based measures of brain microstructure, as well as the influence of alcohol use on this association. MTBI was not associated with any of the MRI-based measures when compared to the no injury group. Unexpectedly the main findings showed that OI was associated with shorter T1 and T2 relaxation time, and higher MTR and MWF in cortical white matter. I then explored the possibility that the unusual results are due to reverse causality or collider bias.

Reverse causality implies that the results are due to another factor that is a result of brain microstructure and that is causally linked to OI. In the full cohort,

reporting one antisocial activity at age 8 was predictive of sustaining an OI, but not mTBI, at age 14 to 16 years. However, the evidence was weak and the differences in brain microstructure seem unlikely to be explained by antisocial activities in this sub-study given the low number of individuals who committed one or more antisocial activities in this subsample.

The unexpected findings may also be the result of a type of selection bias called collider bias. This occurs when two variables independently influence a third variable and that variable is then conditioned upon (Munafò et al., 2018). In this case, having a mTBI or OI increased the chances of participating in the sub-study; if the participants' brain microstructure also influenced participation, then sub-study participation would have been conditioned upon. Collider bias could have induced a spurious association between OI and MRI-based measures of brain microstructure. Additionally, this could have led to a null association between mTBI and MRI-based measures. This is assuming that there is a true association between mTBI and MRI-based measures that has been biased towards the null, while in the same direction, the association between OI and MRI-based measures was biased away from the null. I was unable to explore the influence of participants' brain microstructure on sub-study participation as this was unknown in the full cohort. One approach to overcome this would be to use a genetic variant associated with the MRI-based measures as a proxy for brain microstructure. Unfortunately, I was unable to locate a genetic variant associated with any of the MRI-modalities or brain regions explored in the current study. In order to successfully use a genetic variant associated with an alternative neuroimaging measure, the genetic variant would have to be highly predictive of that neuroimaging measure, which in turn would have to be highly correlated with

the MRI-based measures used here. Potentially suitable genetic association studies of brain structure have focussed on MRI-based measures derived from diffusion tensor imaging studies and brain volume measures, and none have located a genetic variant that is highly predictive. For example, even in a recent genome-wide association study (GWAS) in UK Biobank database explored 3,144 image-derived phenotypes of brain function and structure none of the modalities and brain regions from the current study were included (Elliott et al., 2018).

MTBI was not associated with the MRI-based measures investigated in the current study when compared to participants with no injury. Research using these measures to explore mTBI is limited, and to my knowledge, T1 and T2 relaxation times have not been utilised in the field before. Although the possibility that collider bias has induced a null association cannot be ruled out, the lack of association is in support of previous research. Previously, Narayana and colleagues found no difference in MTR between participants with a mTBI and those with an OI scanned 24 hours post-injury, additionally they reported no difference within-subjects when they scanned again 90 days post-injury (Narayana et al., 2015). There has been only one study using MWF to investigate mTBI. In 11 university ice hockey players who sustained a concussion, there were several clusters with decreased MWF 2 weeks post-injury and no evidence for decreased MWF at 2 months post-injury relative to a baseline scan (Wright et al., 2016). The time post-injury in the current study was between 2 and 7 years, so it is possible that any decrease in MWF following a mTBI would have resolved in this time. Likewise, previous research has failed to establish an association between mTBI and grey matter.

4.4.1 Strengths and limitations

In most of the literature participant samples have had either sports-related concussion (Churchill et al., 2017a), blast-related mTBI in the military (Miller et al., 2016), or have been recruited from emergency departments (Li et al., 2016) or outpatient clinics in hospitals. The use of self-report in a representative sample is a strength in assessing the effects of mTBI in the general population as it is likely that there is a considerable amount of mTBI that goes unreported in medical settings (Cassidy et al., 2004). Using self-report measures in a neuroimaging study is also novel. The potential for selection bias in the current sample is a key limitation, future research will be needed to verify the presence of collider bias and simulations could help.

4.4.2 Chapter summary

I aimed to explore the association between mTBI and brain microstructure using four different MRI-based measures of white and grey matter. I had intended on exploring the influence of alcohol on any observed association between mTBI and MRI-based measures, however the main findings were too unusual to proceed with this analysis. Overall, the main finding that OI was associated with global and lobar MRI-based measures of white matter was unexpected. While this could be a chance association, I speculate that collider bias has occurred based on the evidence that sustaining an OI or mTBI predicted participation in the sub-study. Evidence that brain microstructure also predicted sub-study participation would be needed to confirm this however; if an appropriate genetic variant becomes available then using a genetic variant as a proxy for the MRI-based measures in the current study could provide this evidence. In the next chapter I continue to investigate the effect of mTBI on brain microstructure, using a different MRI modality in players of high contact sport.

Chapter 5 Diffusion tensor imaging study of mTBI in university rugby players

5.1 BACKGROUND

In Chapter 4, I investigated the association between mTBI and four MRI-based measures, the findings were unexpected and possibly due to collider bias or reverse causality. In this chapter I expand on this by utilising MRI data to investigate the effect of mTBI on brain microstructure, using diffusion tensor imaging (DTI) in players of high-contact sports. MTBIs were diagnosed by a physiotherapist and DTI is the most commonly adopted MRI approach in the mTBI literature.

In sport mTBI is known as concussion; sport is a major cause of mTBI with an estimated 1.6 to 3.8 million sport-related mTBIs sustained annually (Langlois et al., 2006). Using athletes to investigate mTBI has benefits as the groups tend to be similar in terms of age, education level and level of physical activity. Ice hockey, rugby union and American football have the highest incidences of mTBI among both adult (Koh, Cassidy, & Watkinson, 2003) and youth (Pfister, Pfister, Hagel, Ghali, & Ronksley, 2016) athletes.

Arguably, sport-related mTBI is the area where research into the effects of mTBI is most applicable as athletes in high contact sports are exposed to repetitive head trauma throughout their careers (for a review see (Zetterberg et al., 2018)). This exposure has been linked with chronic traumatic encephalopathy (CTE), a type of progressive neurodegeneration which requires a post-mortem diagnosis (Pan et al., 2016). Not all individuals exposed to repetitive head trauma

will develop CTE, although this seems to be a necessary exposure for the development of the condition (Pan et al., 2016). A recent investigation of deceased players of American football found neuropathological evidence of CTE in 177 out of 202 players (Mez et al., 2017); the study included a detailed retrospective informant clinical interview providing evidence for the progressive nature of the disease. The authors note that the sample may not be representative of all American football players due to the high number of former college and professional players in the brain bank; however, this was the largest study of this nature to date and the players in the study were exposed to similar types of head trauma during their lives.

There has also been concern that the phenomenon of heading the ball in soccer may also result in deleterious effects (Rutherford, Stephens, & Potter, 2003). Meanwhile continued media coverage of legal disputes between former professional American football players with the national governing body of the sport (National Football League: NFL), as well as personal stories emerging from former rugby and soccer players further highlight the importance of research in this area.

Rugby union (hereafter "rugby") is a popular full-contact sport that is associated with a high incidence of mTBI (Gardner, Iverson, Williams, Baker, & Stanwell, 2014). A report by the Rugby Football Union (RFU) in England reported that mTBI is the most common injury at the professional level, accounting for 17% of all match injuries (England Professional Rugby Injury Surveillance Project Steering Group, 2016). Worldwide, rugby is played by almost 5 million people, and by over 2 million people in the United Kingdom (http://www.worldrugby.org/development/player-numbers). Although previous

studies have included rugby players (Churchill et al., 2017b, 2017a; Murugavel et al., 2014), there are very few studies focussed solely on rugby. A recent study examined prefrontal white matter tissue and metabolites in female rugby players after seasons of play; all players were scanned before and after each season while those with a mTBI were also scanned 24 to 72 hours, 3 months and 6 months post-injury (Schranz et al., 2018). While there were no changes in DTI measures in the mTBI players, an increase in fractional anisotropy (FA) and decrease in radial diffusivity (RD) was observed in the non-TBI players. All players showed decreases in glutamine and this decrease was correlated with FA and RD in the non-TBI players. The authors interpreted the association between metabolite change and DTI measures in the non-TBI group as reflecting recovery processes, while the change in metabolites in the absence of DTI changes in the mTBI group was suggested to represent altered oxidative metabolism (Schranz et al., 2018).

In this study I explored the effect of sustaining a recent mTBI compared to playing a season of rugby without sustaining a mTBI in male university rugby players. The study ran for one university season and I used DTI to explore differences in brain microstructure.

5.2 METHOD

5.2.1 Participants

Participants were recruited from the men's university rugby club at the University of Bristol. Throughout the season, the head coach and team physiotherapist identified players who sustained a concussion during match play and training using the RFU "recognise and remove" guidelines. According to

these guidelines, anyone with suspected sport-related mTBI, either from mechanism of injury or signs and symptoms or both, is removed from play. These players then follow the return to play protocols, which means a graduated return to play following a rest period of 19 days with no training or playing. Players identified as having mTBI had their contact details forwarded to me and I then invited these individuals to have an assessment within 28 days of the injury. Individuals who met the following exclusion criteria could not participate; non-native English speaker; history of neurological disease or severe TBI; contraindications for MRI; claustrophobia that would prohibit MRI; and uncorrected visual or auditory impairment that would impede performance on the tasks or completion of questionnaire measures. The recruitment process for the non-TBI group was through convenience sampling; at the end of the season the captain of the rugby club circulated a message to all players inviting them to take part if they did not sustain a mTBI during the 2017/18 season.

5.2.2 Questionnaire Measures

All questionnaire measures were administered prior to the MRI acquisition. The clinical assessment and preparation for the scan took approximately 30 minutes.

5.2.2.1 Concussion Assessment

A semi-structured interview was administered to participants on the test day to gather information on TBI history, including the most recent mTBI. Participants were provided with the definition of a mTBI and then asked about their history of mTBI. Details on the presence and duration of symptoms, medical

attention sought, and age at injury was gathered. The interview schedule can be seen in appendix 5.1.

5.2.2.2 Sport concussion assessment tool 5th edition (SCAT5)

The SCAT5 was used to evaluate athletes on cognition, balance and post-concussive symptoms. The cognitive domains assessed include immediate and delayed memory, orientation and concentration. It is used as a side-line assessment of injured athletes as well as a baseline assessment before a playing season.

5.2.2.3 Mental speed and switching attention

The Trail-Making Test (TMT) (Reitan, 1958) is a pencil and paper task, participants are given a sheet of paper with 25 circles distributed on the sheet. In part A, the circles are numbered from 1 to 25, and participants are required to connect the circles in ascending order as a measure of mental speed. In part B, the circles are numbered from 1 to 13 and include letters A to L; participants connect the circles in an ascending pattern alternating between letters and numbers (i.e., 1-A-2-B-3-C). Part A measures mental speed, while part B measures switching attention. In both parts, participants were instructed to connect the circles as quickly as possible without lifting the pencil from the paper. Scoring is based on the time in seconds, it takes the participant to complete each part; a longer time is related to greater impairment.

5.2.2.4 Alcohol Use

The Alcohol Use Disorder Identification Test (AUDIT) (Saunders, Aasland, Babor, De La Fuente, & Grant, 1993) was used to examine level of alcohol use and possible misuse. It is a 10 item scale, with good internal

consistency (Cronbach's α = .93) and in over 90% of cases problem drinkers were correctly classified (Saunders et al., 1993). Questions are scored from 0 to 4 with a range of possible scores between 0 and 40. The scale is designed to identify persons with hazardous levels of drinking, hazardous drinking was defined by the authors as a level of drinking above which intervention is preferable to no intervention.

5.2.2.5 Premorbid intellectual functioning

The National Adult Reading Test (NART) consists of 50 words with atypical phonemic pronunciation. Participants are presented with the list of words and required to read each word aloud.

5.2.3 Diffusion Tensor Imaging

Diffusion is thermally-driven random motion. In a barrier-free vessel such as a glass of water, water molecules freely diffuse in all directions, this is known as isotropic diffusion. However, the movement of water in white matter of the brain is said to be anisotropic because it is restricted by tissue microstructure. Water molecules in white matter will diffuse quicker parallel to the long axis of a fibre bundle and slower perpendicular to it. In diffusion MRI a strong magnetic field gradient is applied along a direction x and a signal attenuation will be observed in particles if they diffuse along x compared with an acquisition where no magnetic field gradient was applied. Multiple directions are used so that a three-dimensional diffusion model, known as the diffusion tensor, can be estimated.

A diffusion tensor is a 3 x 3 matrix that has three orthogonal (mutually perpendicular) directions, or eigenvectors, and three positive strengths of

diffusion, or eigenvalues. The major eigenvector indicates the axis of the fibre tract in anisotropic fibrous tissues (O'Donnell & Westin, 2012). The three eigenvalues (λ1, λ2, λ3) denote the strength of diffusion in each of the three eigenvectors. Figure 5.1 shows an isotropic vector (A), where all three eigenvectors are approximately equal and an anisotropic vector (B) where one eigenvector is greater than the other two. Using the diffusion tensor, it is possible to estimate the principal fibre orientation in the brain and generate a colour coded image as in Figure 5.2 below.

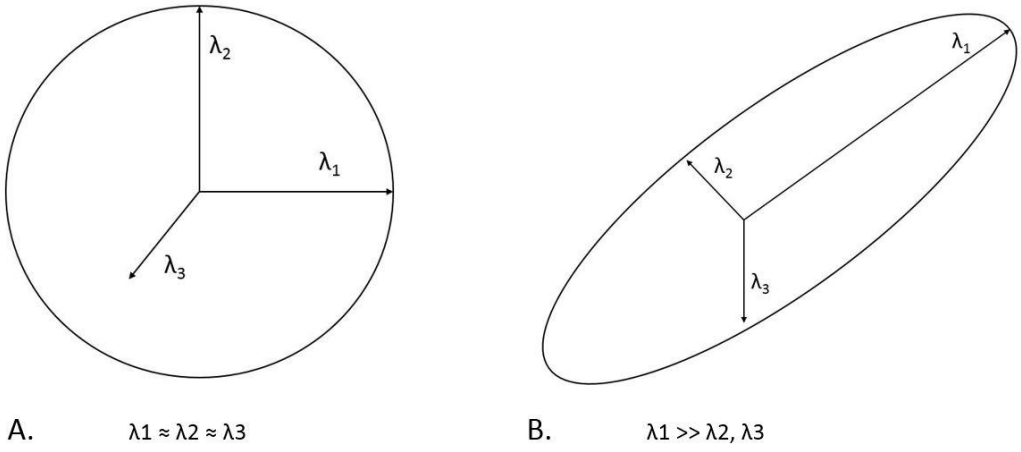

Figure 5-1 Isotropic (A) and anisotropic (B) voxels

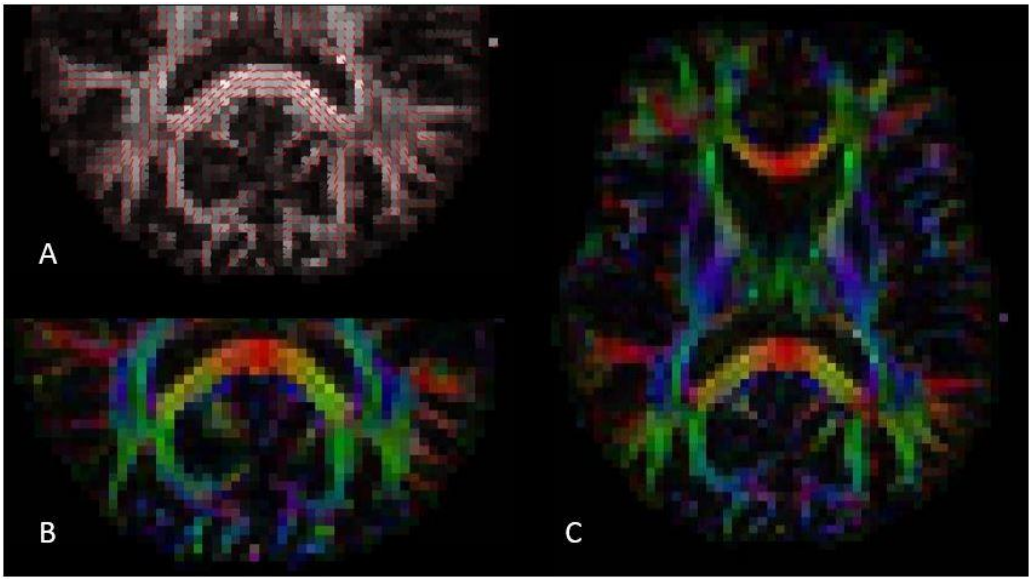

Figure 5-2 shows the estimates of the principal fibre orientation in white matter based on the direction of maximum diffusivity in anisotropic voxels (A). Images with the standard red-green-blue colour coding can be seen in B and C; red indicates left-right orientation, green indicates anterior-posterior orientation, and blue indicates superior-inferior orientation

Fractional anisotropy (FA) is an index of the characteristic of water to move faster parallel and slower perpendicular to the fibre. It is a fraction of the diffusion that is anisotropic, or how the tensors shape differs from a perfect sphere, and it ranges between 0 and 1. FA is the normalised variance of the eigenvalues and is calculated using the following equation:

$$FA = \sqrt{\frac{(\lambda_1-\lambda_2)^2+(\lambda_2-\lambda_3)^2+(\lambda_1-\lambda_3)^2}{2(\lambda_1{}^2+\lambda_2{}^2+\lambda_3{}^2)}}$$

Mean diffusivity (MD) is the molecular diffusion rate and it is the mean of the three eigenvalues ($\lambda1+ \lambda2 + \lambda3 / 3$). FA is affected by the orientation, packing and density of fibres. Additionally, the observed FA and MD may indicate different scenarios. Higher FA and lower MD suggests more restricted movement of water molecules and could indicate either a higher density of fibres or oedema (swelling). While lower FA and higher MD suggests freer movement of water molecules and could indicate either myelin loss or cell death. Other parameters that are generated from DTI include axial and radial diffusivity. Axial diffusivity

(AD) corresponds to water diffusion parallel to the fibre, it is the principal eigenvalue, λ1, and is considered a measure of axonal content. Radial diffusivity relates to water diffusion perpendicular to the molecular rate, it is the average of the second and third eigenvalues (λ2 + λ3 / 2) and is considered a measure of myelin content.

5.2.4 MRI Acquisition

All MRI scanning and assessments were carried out at the Clinical Research Imaging Centre (CRiC) at the University of Bristol. Images were acquired on a 3T MRI scanner (Siemens Skyra) with a 32 channel receive-only head coil. A high resolution (0.9 × 0.9 × 0.9mm) T1-weighted 3D volume scan was acquired for each participant using the MP-RAGE sequence with the following parameters: repetition time (TR) = 1800ms; echo time (TE) = 2.25ms; field of view (FOV) = 240mm; slice thickness = 0.9mm; flip angle = 90 °. The diffusion weighted imaging sequence was acquired on 59 slices along the anterior-posterior direction with the following parameters: resolution = 2.7 × 2.7 × 2.7mm; TR = 8500ms; TE = 92ms; FOV = 350mm; slice thickness = 2.7mm; 64 directions; b = 0 and b = 1000s/mm^2.

5.2.5 Image Processing

Image pre-processing and statistical analysis was performed with FSL [v. 5.0.8; Oxford Centre for Functional MRI of the Brain; FMRIB (Smith et al., 2004)]. Diffusion data was processed using the FMRIB's Diffusion Toolbox (FDT). The data was first corrected for eddy currents and motion using the eddy tool. The fieldmap was then used to correct for distortion. The fieldmap was converted from radians per second to hertz per second and smoothed using

FUGUE, a conversion matrix, obtained from registering the brain extracted magnitude image to the b0 image, was used to register the smoothed fieldmap to the b0 image with FUGUE. Finally, the eddy corrected dataset was corrected for distortion using the smoothed, registered fieldmap in hertz. The corrected diffusion images along with a brain extracted binary brain mask and gradient directions were fed into DTIFit to generate three pairs of eigenvalues, and eigenvectors, an FA image and an MD image. AD was the principal eigenvalue ($\lambda 1$) and RD was calculated as the average of the second and third eigenvalues ($\lambda 2 + \lambda 3 / 2$).

5.2.6 Statistical Analysis

Whole-brain voxel-wise statistical analysis of the diffusion image data was carried out using TBSS (Tract-Based Spatial Statistics) (Smith et al., 2006). All 13 subjects' FA data were aligned into a common space using the nonlinear registration tool FNIRT (Andersson, Jenkinson, & Smith, 2007a, 2007b), which uses a b-spline representation of the registration warp field (Rueckert et al., 1999). Next, the mean FA image was created and thinned to create a mean FA skeleton which represents the centres of all tracts common to the group. The WM skeleton was then thresholded at FA > 0.20 to exclude areas of high between-subject variability in the minor tracts. Each subject's aligned FA data was then projected onto this skeleton. For MD, RD and AD, the data was first aligned using the same nonlinear registration as above, then all participants warped data was merged and then projected onto the mean FA skeleton.

Statistical inference was made via permutation testing with FSL's RANDOMISE tool and threshold-free cluster enhancement (TFCE) to avoid choosing an arbitrary threshold for cluster-forming and also for multiple comparison correction (Smith & Nichols, 2009); 1716 permutations were run as this was the maximum for my data. Two contrasts were performed at the group level for each diffusion measure (non-TBI > recent mTBI and non-TBI < recent mTBI) and were considered significant at the $p<0.05$ level. Analyses were not adjusted for age or sex as all players were a similar age and the same sex, nor for additional confounders due to the small sample size. The anatomical location of each significant cluster was identified from the John Hopkins University ICBM-DTI-81 white-matter labels atlas (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005) using AUTOAQ for automated anatomical labelling of activated clusters (Winkler, 2012), and an arbitrary threshold of > 5% probability to determine regions contained within each cluster.

Questionnaire measures were analysed using analysis of variance (ANOVA) for continuous outcomes and chi square for categorical outcomes to test for differences between the two groups.

5.3 RESULTS

5.3.1 Participants

We assessed seven players with recent mTBI (age range 19 to 22 years; mean 19.94, SD 1.28) an average of 42 days post-injury (range 9 to 132 days); there was an additional one player who sustained a mTBI but was unable to participate due to MRI contraindications. Seven players (age range 18 to 22 years,

mean 20.82, SD 1.11) who did not sustain a mTBI during the 2017/18 season were also assessed an average of 43 days after the final match of the season (range 41 to 44 days). Due to the high prevalence of mTBI in rugby players it was expected that those who had not sustained a mTBI in the previous season may have some history of head injury, four of the seven non-TBI players had some history of head trauma. Two players reported having stitches in their head below the age of 10 years, while one player had sustained a mTBI playing rugby approximately 5 years previously. These three players were included in the analysis; however, one player was excluded from analysis as he had experienced several sport-related mTBIs in the two years before assessment. The recent mTBI group performed better on the concentration subtest of the SCAT5 and completed TMT part B in a faster time than the non-TBI group, see Table 5.1. For details of the most recent concussion in each of the groups see Tables 5.2 and 5.3.

Table 5-1 Descriptive Statistics for neuropsychological assessment and questionnaire measures

	MTBI (n=7) *M (SD)*	Non-TBI (n=6) *M (SD)*	F-statistic	P-value
Mean age	19.94 (1.28)	20.86 (1.21)	1.77	.210
Previous mTBIs	2 (0.58)	0.5 (0.55)	22.85	< 0.001
SCAT5				
Symptom score	1.57 (2.44)	1.67 (2.25)	0.01	.943
Symptom severity	2.14 (3.53)	2 (3.03)	0.01	.939
Orientation	5 (0)	4.67 (0.82)	1.18	.300
Concentration	4 (0.82)	3 (0.63)	5.92	.033
Immediate memory	22.57 (3.46)	21.33 (1.51)	0.66	.435
Delayed memory	7.67 (2.25)	6.5 (0.84)	1.42	.262
Balance	0.71 (1.11)	2 (3.03)	1.10	.316
TMT				
Part A	22.43 (6.83)	24.83 (5.38)	0.48	.501
Part B	42.43 (10.91)	61 (16.09)	6.11	.031
AUDIT	14.86 (4.56)	11.83 (3.06)	1.89	.196
NART Errors	14.86 (3.39)	21.67 (6.19)	6.33	.029

Note: SCAT5: Sport Concussion Assessment Tool 5th Edition; TMT: Trail-making Task; AUDIT: Alcohol Use Disorder Identification Test; NART; National Adult Reading Test

Table 5-2 Details of most recent sport-related mTBIs sustained by the players in the recent mTBI group

Participant	Days since injury	Symptoms at most recent injury	No. of mTBI in lifetime
1	30	Unable to speak; felt emotional	2
2	51	Dazed/feeling in a fog; nausea the next day	2
3	16	LOC for a few seconds; feeling in a fog; confusion and disorientation; pins and needles in lower leg (physiotherapist thought this was because of how he landed); mild headache for the first week after	1
4	9	saw stars; numerous hits so he was taken out of play; slight headache	4 or 5
5	9	PTA for 6 or 7 hours, with some fleeting images; repeating the same conversation for a few days after; confusion for a few days after	2
7	132	LOC for a few seconds; nausea and vomiting for one week after; headache for one week after	2
14	49	5 mins PTA; seeing white flashes; stumbling; time moving weirdly	2

Table 5-3 Details of most recent mTBIs sustained by the players in the non-recent mTBI group

Participant	Days since last match	Age at most recent mTBI	Details of most recent mTBI	No. of mTBI in lifetime
6	41	.	n/a	0
8	42	.	n/a	0
9	44	4 or 5	Hit head on radiator; received stitches as an outpatient	1
10*	44	19	Sustained while playing rugby; memory problems for duration of match; déjà vu after the match	6
11	44	8	Hit head on a wall while cycling; received stitches as an outpatient	1
12	44	14 or 15	Sustained while playing rugby; LOC for a few seconds; disoriented for a few minutes; feelings of nausea	1
13	44		n/a	0

* This participant was excluded from analyses because of the high number of lifetime mTBIs

5.3.2 Tract-based Spatial Statistics

Two clusters showed lower MD in recent mTBI players compared to non-TBI players (non-TBI > recent mTBI contrast; Figure 5.3 A). The largest cluster was located in the body of the corpus callosum, extending into the superior corona radiata and the superior longitudinal fasciculus in the left hemisphere. The second cluster was located in the sagittal stratum in the right hemisphere.

There were four clusters showing lower AD in recent mTBI players compared to non-TBI players (Figure 5.3 B). The largest cluster was located in the body and genu of the corpus callosum, extending to the superior corona radiata and superior longitudinal fasciculus in the left hemisphere. The second cluster was in the superior corona radiata and superior longitudinal fasciculus in the right hemisphere. The third cluster was in the body and genu of the corpus callosum; and the fourth was in the body of the corpus callosum. See Table 5.4 for details of significant clusters.

Interestingly, there was no clear evidence of differences in either FA or RD between the two groups in either contrast. Furthermore, there was no suprathreshold results for any of the DTI parameters in the second contrast (non-TBI < recent mTBI).

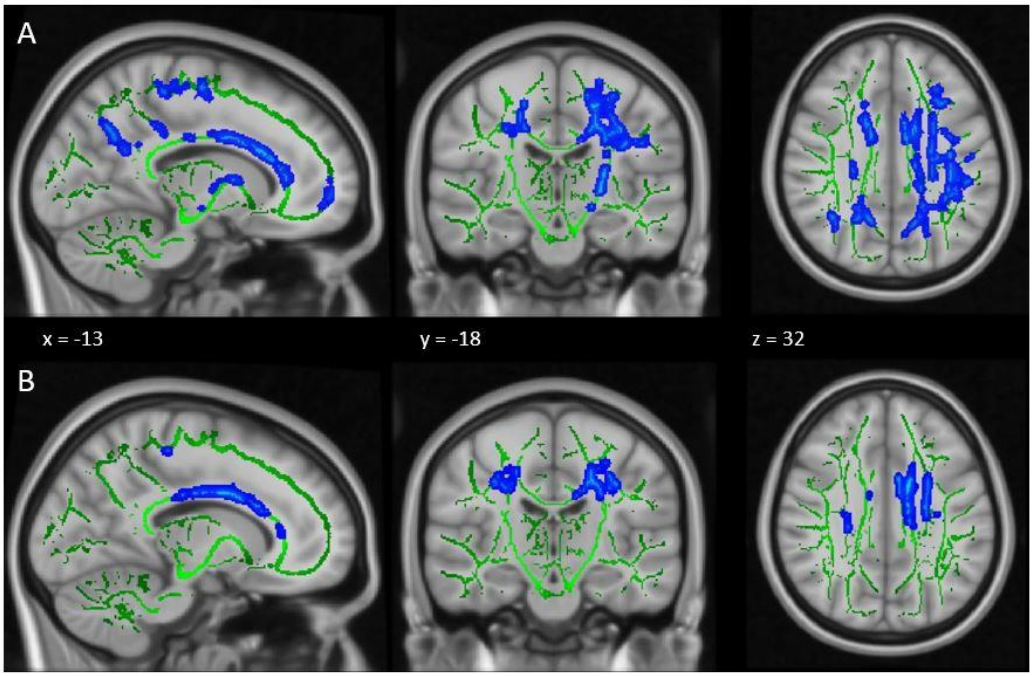

Figure 5-3 Tract-Based Spatial Statistics (TBSS) analysis results identified lower MD (A) and lower AD (B) in players with recent mTBI compared to players with no recent mTBI. Clusters with significant differences have been thickened into local tracts using the TBSS_fill command in FSL shown on the FA skeleton (green). The figure displays data from 13 participants displayed on the MNI 1mm brain template in radiological format on sagittal, coronal, and axial sections.

Table 5-4 Peak voxel in MNI coordinates and number of voxels for brain regions that showed significantly lower MD and AD in rugby players with recent mTBI compared to players without recent mTBI identified using tract-based spatial statistics and threshold-free cluster enhancement

Region	Voxels	Hemisphere	Minimum p value	x	y	z
MD						
Body of corpus callosum, superior longitudinal fasciculus, superior corona radiata	11103	L	.010	-18	-25	36
Sagittal stratum (including inferior longitudinal fasciculus and inferior fronto-occipital fasciculus)	6	R	.050	41	-35	-8
AD						
Body of corpus callosum, superior corona radiata, genu of corpus callosum, superior longitudinal fasciculus	2055	L	.010	-24	-17	34
Superior corona radiata, superior longitudinal fasciculus	426	R	.024	21	-28	44
Body of corpus callosum, genu of corpus callosum	70	L/R	.046	10	19	21
Body of corpus callosum	7	L/R	.048	11	-5	30

5.3.3 Correlating the TBSS findings with the questionnaire outcomes

As an additional exploratory analysis, I correlated the TBSS findings with the questionnaire outcomes to explore how they were associated. I extracted a mean MD value and a mean AD value for each individual from the skeletonised data using a mask of the significant clusters. Then I carried out Pearson's correlations on the mean AD and MD values and the questionnaire outcomes. Following Bonferroni correction, there was a large positive correlation between AD and MD (r = 0.93, 95% CI 0.78 to 0.98, p <0.001). There was also very weak evidence for a large negative correlation between MD and lifetime history of mTBI (r = -0.80, 95% CI -0.94 to -0.45, p = 0.096). There was no evidence for a correlation between any of the other questionnaire outcomes and either diffusion metric.

5.4 DISCUSSION

This study explored recent mTBI in university rugby players using DTI-derived parameters. I found lower MD and lower AD in several clusters in the white matter tract skeleton of players with recent mTBI compared to players without recent mTBI using a whole-brain voxel-wise analysis of DTI data. These clusters were located in the body and genu of the corpus callosum; the sagittal stratum in the right hemisphere; the bilateral superior corona radiata and the bilateral superior longitudinal fasciculus. Surprisingly no differences in FA and RD were observed between groups, and the players with recent mTBI performed *better* on two of the neuropsychological tests.

The lack of evidence for a difference in FA is in contrast with previous studies that have found either higher (Borich, Makan, Boyd, & Virji-Babul, 2013; Churchill et al., 2017a; Henry et al., 2011; Meier et al., 2016; Sasaki et al., 2014) or lower (Chamard et al., 2013; Churchill et al., 2017b) FA following sport-related mTBI. However, in previous research higher FA was often accompanied by lower MD in some of the same regions that showed lower MD in recent mTBI players in the current study, such as the body of the corpus callosum (Henry et al., 2011) and the corona radiata (Churchill et al., 2017a; Sasaki et al., 2014). Borich and colleagues reported higher FA, lower AD and a trend for lower MD in the white-matter skeletons of twelve adolescents assessed an average of 35 days following sport-related mTBI (Borich et al., 2013), which is similar to the average of 42 days post-injury here.

Lower MD suggests restricted diffusion of water molecules in the identified regions; this could indicate oedema (i.e. swelling) as inflammation of fibres may impede diffusion. On the other hand, the lower MD values seen in participants with a recent mTBI is influenced by lower diffusion in the principal direction, i.e. lower AD. AD represents diffusion parallel to the predominant fibre orientation along the axon and changes in AD are thought to be reflective of changes to the axon itself rather than myelin which is better characterised by RD (Song et al., 2002). The lower AD seen in the white matter skeletons of players with recent mTBI here may reflect traumatic axonal injury that occurs in the days postinjury (Henry et al., 2011; Newcombe et al., 2007). Consistent with this notion is histological research showing that the brains of five individuals with concussion, who died of unrelated causes, all had axonal injury (Blumbergs et al., 1994).

There were indications of lower MD and AD in the body of the corpus callosum, the superior longitudinal fasciculus (SLF) and the superior corona radiata in players with recent mTBI in the current study. The corpus callosum is the largest fibre tract in the nervous system; it connects the two hemispheres of the cortex in placental mammals (Aboitiz & Montiel, 2003). There is a rough representation of the different cortical areas along the corpus callosum, such that posterior cortical areas are connected by fibres in the posterior corpus callosum. The body of the corpus callosum contains relatively large, highly myelinated fibres that connect auditory, motor and somatosensory areas of the brain. The SLF and superior corona radiata are less well understood. The SLF extends from the frontal lobe, through the parietal and temporal lobes and arches around the Sylvian fissure (the large sulcus by the temporal lobe). It is a large fibre bundle comprising four components and has been associated with complex processes such as working memory (Karlsgodt et al., 2008) and language (Bernal & Altman, 2010). The superior corona radiata is a white matter bundle with ascending and descending axons. It extends ventrally to the internal capsule and dorsally becomes a fan-like structure. The superior corona radiata is fundamental for motor function.

Damage to any of these major white matter tracts could feasibly lead to post-concussive symptoms such as ringing in the ears, dizziness and sensitivity to light. However, the players in the current study were mostly asymptomatic. Furthermore, it is surprising that the recent mTBI group performed better on two of the neuropsychological assessments - one possible explanation for this is the subject-expectancy effect, whereby participants expect particular results and therefore alter their behaviour to reflect a belief that their behaviour should

change (Supino, 2012); in this case participants with recent mTBI may have believed their performance would be impaired – therefore they may have concentrated more on the tasks.

5.4.1 Strengths and Limitations

Caution must be exercised when interpreting the findings as unfortunately this study has some key limitations, particularly the small sample size and the wide range of days between injury and assessment. The low number of players with recent mTBI likely reflects updates to the sport-related mTBI protocol implemented during the 2017/18 season. Players received explicit training on mTBI identification and avoidance, enhanced physical preparation and technical coaching. Ideally this study would have continued for an additional season to include more participants. Furthermore, the wide range of time post-injury make interpretation of the findings more challenging. The duration between injury and assessment ranged between 9 and 132 days and it is not clear whether microscopic changes associated with mTBI would have resolved differently within this timeframe. Ultimately stringent criteria related to time since injury were not applied in order to maintain participant numbers. Additionally, TBSS is a voxel-based approach which may lack the sensitivity to identify all alterations following a mTBI, and the spatial normalisation needed for the approach can introduce error if inaccurate. Nevertheless, TBSS is useful for exploratory studies where *a priori* regions of interest do not need to be specified, and also for group-based analyses as it is data-driven and fully automated (Henry et al., 2011).

Including players of the same sport with no recent mTBI as controls minimises the possibility that differences in brain structure reflect specialised

training or other selection factors and should be considered a strength of the study. Although some of the control group had previously sustained a head trauma, this is expected given the population and all had incurred injuries at least five years before the assessment; this is similar to previous studies where non-TBI athletes also had some history of mTBI (Meier et al., 2016; Schranz et al., 2018).

5.4.2 Chapter summary

This exploratory study suggests that recent mTBI in male university rugby players is associated with DTI indicators of processes such as axonal injury or oedema not seen following a season of playing rugby. Due to the small sample size, wide range of time post-injury and the cross-sectional design further research is required to explore the effects of mTBI in rugby. Future research should include pre-season scans to use as a baseline measure for each individual and a larger sample size. Nonetheless, the current findings lend support to previous studies of sport-related mTBI showing lower MD in regions such as the corpus callosum and corona radiata. The use of a standard analysis tool, TBSS, will allow for easy comparability with existing research and allow the findings to be included in any future meta-analyses in the area.

Chapter 6 General Discussion

MTBI is an injury to the head caused by blunt trauma or external force that leads to an alteration of consciousness; loss of consciousness may occur for less than 30 minutes and post-traumatic amnesia of less than 24 hours may be present. Estimates for the incidence rate of TBI are usually based on hospital records and other medical records; the rate is believed to be about 235 per 100,000 in Europe with mTBI accounting for approximately 80% of all recorded TBI (Tagliaferri et al., 2006). The true rate of mTBI is assumed to be much higher as not all individuals who sustain a mTBI will seek medical attention. Following mTBI, an individual may experience post-concussive symptoms (PCS), which include physical, cognitive and psychological symptoms. The usefulness of PCS is debated as these symptoms have a high prevalence in the general population and in other disorders such as depression (Rapp & Curley, 2012).

It is thought that any effects of a mTBI in childhood or adolescence may become more evident as the individual navigates the demands of different developmental stages, such as adolescence (Taylor & Alden, 1997). Adolescence is a time of increased risk-taking (Steinberg, 2008) and there is evidence to suggest that this risk-taking behaviour is further heightened in young people with a history of mTBI. The first aim of my thesis, therefore, was to investigate the association of mTBI in youth with risk behaviours using systematic review and epidemiological analysis of behavioural data from a birth cohort study. Furthermore, there is evidence that magnetic resonance imaging (MRI) techniques that assess brain microstructure are sensitive to neuropathology in mTBI (Bigler, 2013). The second aim was to explore the neural substrates of mTBI using

different MRI measures of brain microstructure. In this general discussion I will summarise my main findings in the context of each of these aims and then discuss implications, limitations and future research.

6.1 AIM ONE: INVESTIGATING RISK BEHAVIOUR

6.1.1 Summary of findings

In Chapters 2 and 3 I investigated the association between mTBI in childhood and adolescence and the association with risk behaviour. Risk behaviour was defined as substance use, criminal behaviours and psychiatric symptoms. The systematic review in Chapter 2 provided some evidence for a link between early mTBI and substance use, however this was based on three articles from two studies. There was conflicting evidence in terms of psychiatric symptoms and only one article reported findings linking mTBI with crime. In total only six articles from four studies were eligible for review; therefore, the review served to highlight the paucity of good quality longitudinal evidence for the effects of mTBI in youth. All of the included articles were based on observational studies, which are the most appropriate way of assessing associations with mTBI as it is obviously unethical to inflict head trauma on individuals for research purposes. However, observational studies are unavoidably rated as 'low' quality of evidence based on GRADE criteria. Encouragingly, all included studies used medical records to identify TBI and included a control group of individuals without TBI. However, three of the articles were downgraded to very low quality of evidence. This can be avoided in future by reporting effect sizes and confidence intervals and adequately adjusting for potential confounding.

In Chapter 3 I aimed to build on the findings from my systematic review by carrying out a good quality longitudinal study of an association between mTBI and risk behaviour using data from a birth cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC). It was not possible to use medical records to identify mTBI like previous studies had; instead I used parent- and self-report measures, which may have included cases of TBI where medical attention was not sought. While all of the studies in the systematic review had included one uninjured control group for comparison, I used the novel approach of incorporating a second control group with orthopaedic injuries (OI) to act as a negative exposure control alongside an uninjured control group. MTBI and OI were reported between birth and age 16 years. I compared the three participant groups on seven outcomes of risk behaviour at age 17 years related to substance use (alcohol, tobacco and cannabis); crime (committing offences and being in trouble with the police) and psychiatric symptoms (conduct and peer problems). In support of the studies included in the systematic review, mTBI was associated with all seven outcomes compared to no injury. However, in *both* the comparison with no injury and the comparison with OI mTBI was only associated with hazardous alcohol use. The negative control analysis with OI is intended to adjust for potentially unmeasured confounding around sustaining an injury. Additionally, there is no plausible biological mechanism for an association between OI and risk behaviour (i.e., no brain changes would have occurred as a result of the injury). In other words, the strongest causal evidence was for an association between mTBI in childhood and adolescence and later alcohol use.

Another interesting finding from this study was that participants with an OI had higher odds of committing offences, but not of being in trouble with the

police, in comparison to the no injury group. The mTBI group had higher odds of both crime-related behaviours. This suggests that there could be a common underlying trait for sustaining an injury and for criminal activities, such as sensation seeking, rather than a biological cause related to brain changes from the head trauma. On the other hand, those with mTBI were more likely to get in trouble with the police, which could indicate a vulnerability in those with a head trauma in terms of the criminal justice system.

6.1.2 Implications of findings

MTBI in Chapter 3 was associated with all seven included risk behaviour outcomes relative to an uninjured control group and the review in Chapter 2 indicates a relationship between mTBI and some risk behaviours. The findings therefore support previous cross-sectional (Ilie et al., 2014, 2015; Max et al., 1998) and longitudinal (McKinlay et al., 2014, 2009; Tonks et al., 2011; Winqvist et al., 2007) research that has showed an association between mTBI in youth and later risk behaviour when compared to just one control group. However, the most noteworthy finding from the systematic review was that there is a dearth of good quality evidence for a causal association between mTBI and later risk behaviour. Additionally, all of the included studies relied on the temporal relationship between injury and behavioural outcome to suggest causality. Using just one control group does not preclude the possibility that both mTBI and engaging in risk behaviour have a common causal factor or are both indicative of some underlying trait such as sensation seeking.

I aimed to overcome this limitation by using a negative control analysis in Chapter 3. By including a group with extracranial injuries, it is possible to account

for unmeasured confounding around sustaining an accidental injury. Using this method, I found evidence for a causal effect of mTBI on hazardous alcohol use but not for the other risk behaviours. However, the lack of evidence for an association between mTBI and crime and psychiatric symptoms in the negative control analyses contrasts with two previous studies that used a negative control design on data from Swedish population registers. They found that individuals with a TBI diagnosis at a mean age of 24 years had higher odds of committing violent crime compared to participants with epilepsy, participants from the general population and in sibling control studies (Fazel et al., 2011). Additionally, participants with a TBI diagnosis had a higher risk of psychiatric inpatient admission and psychiatric outpatient visits, as well as four other adverse life outcomes, compared to participants with non-TBI fall-related injuries, participants from the general population and sibling controls (Sariaslan et al., 2016). Neither study was included in my systematic review because of the age range of participants (Fazel et al., 2011) or time of publication (Sariaslan et al., 2016). The older sample and use of official records in the Swedish population studies may account for the contrasting findings in relation to crime and psychiatric symptoms. Fazel and colleagues noted lower rates of violent crime among participants aged 16 years or younger at TBI diagnosis (Fazel et al., 2011), while in my study cases of mTBI were sustained at age 16 years or younger. Also, psychiatric symptoms in ALSPAC were based on questionnaires that may provide a sub-clinical diagnosis where recorded at age 17, Sariaslan and colleagues recorded the outcomes from age 26 years onward and psychiatric outcomes were based on national registries (Sariaslan et al., 2016). Additionally, the larger sample sizes in

the Swedish population studies increase the ability to detect small effects that may nevertheless be important at a population level.

Overall, while these two chapters extend the literature showing an association between mTBI in youth and later risk behaviour, the review highlighted the scarcity of supporting evidence and in Chapter 3 I have only provided evidence for a causal association between mTBI and alcohol use. Much more research is needed to draw firm conclusions about the connection between a mTBI that is sustained as a child or adolescent and the propensity for engagement in risk behaviour. Future research should continue to use an additional injury group to uncover potentially unmeasured confounding as a way to strengthen causal inference.

6.2 AIM TWO: INVESTIGATING BRAIN STRUCTURE

6.2.1 Summary of findings

In Chapters 4 and 5 I investigated the association between mTBI and brain microstructure using a variety of MRI techniques. The MRI data for Chapter 4 was collected as part of a sub-study exploring axons, testosterone and mental health in 507 male participants from ALSPAC. Similar to Chapter 3, I included a negative exposure group (OI) as well as an uninjured control group. MRI-based measures of grey and white matter in the four lobes and whole cortex included magnetisation transfer ratio (MTR), myelin water fraction (MWF), T1 relaxation time and T2 relaxation time. All of these measures have been used infrequently in the mTBI literature making this quite a novel study. However, the findings were unexpected: there was no evidence for an association between mTBI and MRI-

based measures when compared to the no injury group. The main outcome was that OI was associated with shorter T1 and T2 relaxation time and higher MWF and MTR in white matter when compared to both the no injury and mTBI groups. Shorter T1 and T2 relaxation times indicate a lower presence of water or that the movement of water is being more obstructed by a greater presence of brain microstructure. While higher MWF and MTR suggests more myelinated axons by indication of more water in myelin bilayers and more myelin-bound water respectively.

There is no plausible biological mechanism for this association with OI which suggests that there was some kind of bias in the data. The evidence for reverse causality was very weak – reporting one antisocial activity, but no more than one, was weakly associated with OI in the whole cohort. Another possibility is that a form of selection bias, known as collider bias, may be operating. This is when two variables (i.e. having an injury and brain microstructure) influence a third (i.e. participation in the sub-study), thereby inducing a spurious association between the two variables. In this case, I would assume that there was a true association between mTBI and MRI-based measures which has been biased towards the null, while the true null association between OI and MRI-based measures has been biased in the same direction away from the null. Sustaining an injury was associated with higher odds of participation in the sub-study; however, providing evidence that brain microstructure was influencing participation was not straightforward as there was no measure for brain microstructure for the full birth cohort. A genetic variant associated with the included MRI-based measures or brain regions could act as a proxy for brain microstructure in the whole cohort. Unfortunately, there is no suitable genetic variant currently available. For genetic

variants associated with other MRI measures of white matter to be usable, the variants would have to first be highly predictive of the MRI measure with which they are associated and second, this MRI measure would have to be highly correlated with the measures included in my study. I was unable to locate such a suitable genetic variant. Even in a genome-wide association study of over three thousand image-derived parameters, the MRI-techniques and brain regions from Chapter 4 were not included (Elliott et al., 2018).

Finally, in Chapter 5, I aimed to assess brain microstructure using diffusion tensor imaging (DTI), a more widely used MRI technique. I conducted a case-control analysis on university rugby players. Rugby is a high-contact sport with a high rate of mTBI and few studies investigating sport-related mTBI have focussed solely on rugby players. DTI is a measure of the diffusion of water molecules restricted by brain microstructure and there are four main parameters derived from DTI. Fractional anisotropy (FA) is an index of the characteristic movement of water (faster parallel and slower perpendicular to axonal fibres); mean diffusivity (MD) indicates the molecular diffusion rate; radial diffusivity (RD) indicates water diffusion perpendicular to the axonal fibres and axial diffusivity (AD) indicates water diffusion parallel to the axonal fibres.

I carried out this study in one university season by collaborating with the University of Bristol men's rugby club. The team coach and physiotherapist notified me when a player sustained a mTBI, which was diagnosed by the physiotherapist, and I then invited that player to participate. At the end of the season, the club captain circulated a message on my behalf inviting players who did not sustain a mTBI to take part. In total I analysed data for seven players with recent mTBI and six without recent mTBI. Several clusters in the white matter

skeletons of players with recent mTBI had lower MD and lower AD compared to players without recent mTBI. The findings could indicate axonal injury or oedema in the recent mTBI group in brain regions that have been cited in previous research, such as the body of the corpus callosum (Henry et al., 2011) and the corona radiata (Churchill et al., 2017a; Sasaki et al., 2014). However, caution must be exercised when interpreting the findings as the sample size was quite small and there was a very wide-range of time post-injury.

6.2.2 Implications

In Chapter 4, there was a lack of association between mTBI and MRI-based measures 4- to 7-years post-injury and in Chapter 5 there was evidence of neuropathology in the recent mTBI group an average of 42 days post-injury. Assuming the former represents a true null association as opposed to an artefact of bias, and notwithstanding the limitations of the latter, the findings from these two chapters could indicate that brain changes following mTBI are present in the days and weeks following injury but not in the longer term. However, even while overlooking the possibility of collider bias in Chapter 4, it is still difficult to unite the findings from the two chapters due to the different MRI modalities used.

Previous studies using MTR (Narayana et al., 2015) and MWF (Wright et al., 2016) did not reveal differences in participants with mTBI in comparison to controls when assessed just 2 to 3 months post-injury. On the other hand, there is evidence that DTI can be sensitive to mTBI even when performed years post-injury. For example, Inglese and colleagues found lower FA in 20 participants 4 days post-mTBI and 26 participants 5.7 years post-mTBI compared to 29 age- and sex-matched controls in several regions of interest using DTI (Inglese et al.,

2005). Furthermore, they reported no difference between the two mTBI groups. Elsewhere, athletes with mTBI were reported to have higher FA and lower MD 26 months post injury (Churchill et al., 2017b).

DTI is a useful tool for exploring brain microstructure, but it has some limitations. The method works best in fibre bundles with one major orientation as the tensor is unreliable in regions where fibres cross or 'fan' apart (O'Donnell & Westin, 2012). It is unsurprising therefore, that differences between mTBI and non-mTBI participants are usually located in large fibre bundles that are restricted along a single orientation and relatively unimpeded by crossing fibres, such as the corpus callosum and the superior longitudinal fasciculus (Aoki & Inokuchi, 2016). Additionally, the interpretation of DTI measures is not straightforward as high FA and low MD may indicate healthy tissue with a high density of fibres or oedema; while low FA and high MD could indicate either cell death or myelin loss. This means that any DTI findings related to mTBI can be interpreted as damage to the brain without being considered spurious. This is evidenced in the literature where higher FA (Borich et al., 2013; Churchill et al., 2017b; Henry et al., 2011; Meier et al., 2016; Sasaki et al., 2014); lower FA (Chamard et al., 2013; Churchill et al., 2017a; Veeramuthu et al., 2015) or just higher MD (Cubon, Putukian, Boyer, & Dettwiler, 2011; Narayana et al., 2015) have been reported as indicating brain damage among participants with mTBI. Null findings for an association between mTBI and DTI parameters are rarely reported unless another measure such as magnetic resonance spectroscopy (Schranz et al., 2018) is included in the paper. Despite the limitations of DTI, it has been widely employed in the field for many years. A recent systematic review of 86 studies concluded that although DTI is sensitive enough to detect differences associated with mTBI, it lacks specificity

and the authors suggest a more standardised approach to DTI studies of mTBI in future (Asken, DeKosky, Clugston, Jaffee, & Bauer, 2018)

Other promising methods for the investigation of mTBI were used in Chapter 4. MWF, T1 and T2 relaxation which have been found to be more highly correlated with histological data than DTI parameters (Björnholm et al., 2017). MWF is a relatively new method and it has so far been utilised in just one study of mTBI (Wright et al., 2016). Analysing and processing MWF data requires some specialist expertise and hopefully it will become more streamlined and therefore easily accessible soon. Future research should integrate different MRI approaches and collect data at multiple timepoints to better understand the neuropathological processes in the days, months and years following mTBI.

6.3 LIMITATIONS AND FUTURE DIRECTIONS

This thesis has provided evidence that sustaining a mTBI in youth is associated with later hazardous alcohol use and with indications of axonal injury or oedema using specific MRI techniques. Addressing the question of how these two factors relate to one another was beyond the scope of my thesis but would be an excellent avenue for future research. MTBI causes a complex cascade of chemical changes that can lead to diffuse axonal injury away from the site of impact that may be evident from hours to weeks after injury (Giza & Hovda, 2001) and can result in impaired cognitive and motor functioning. I can suggest three ways that altered functioning as a result of the head trauma could lead to later engagement in risk behaviour.

First, deficits in cognitive functioning following injury could make the individual struggle with their usual activities such as schoolwork and

extracurricular interests. In a study of 1,897 high school students, negative attitudes towards school and poorer academic achievement were associated with substance use at age 14 years (Bryant, Schulenberg, O'Malley, Bachman, & Johnston, 2003). Without adequate support following mTBI, adolescents may then disengage with academic activities and instead engage with risk behaviours, such as alcohol use. Second, emotional problems which are associated with alcohol use may arise as a result of the altered level of functioning following mTBI and this could lead to increased risk behaviour. For example, there may be a decline in self-esteem as the individual struggles to perform to preinjury levels (Bennett & Raymond, 1997) and low self-esteem has been associated with increased substance use in high school students (Scheier, Botvin, Griffin, & Diaz, 2000; Wild, Flisher, Bhana, & Lombard, 2004). Finally, impaired executive function could directly contribute to increased engagement in risk behaviour following mTBI through mechanisms that have been associated with problematic substance use such as inhibitory control (Nigg et al., 2006; Verdejo-Garcıá, Lawrence, & Clark, 2008).

As mentioned, formally exploring these possibilities was beyond the scope of this thesis. However, in Chapter 5 I found evidence for neuropathological processes such as axonal damage or oedema in the body and genu of the corpus callosum, superior corona radiata and the superior longitudinal fasciculus. All three of these regions are large fibre bundles that are fundamental for efficient complex neuronal functioning and damage to these white matter tracts could disrupt cognitive functioning. Surprisingly, the only differences in measures of cognition I observed between participants with and without recent mTBI in Chapter 5 showed poorer performance in the group without recent mTBI. I

suspect this has to do with subject-expectancy where the recent mTBI group exerted more effort to overcome the expectation that their performance might be impaired following mTBI. On the other hand, previous studies that have reported poorer cognitive function in rugby players have compared them to players of non-contact sport (Hume et al., 2016; Shuttleworth-Rdwards & Radloff, 2008). Similar to my study, Thornton and colleagues compared rugby players with self-reported concussion to rugby-playing participants who did not report heavy concussion and they found no difference in neuropsychological functioning (Thornton et al., 2008).

This raises the issue that the use of a rugby playing group could lead to residual confounding as there is some speculation that high-contact sports such as rugby also cause more repetitive sub-concussive head trauma that may have a cumulative effect on brain function. An impact of 98g is the threshold for symptomatic mTBI for 75% human tolerance (Bailes, Petraglia, Omalu, Nauman, & Talavage, 2013) (g refers to the natural unit of acceleration, often rounded to 9.8 metres per second squared, and standing in normal gravity there is a force of 1g). However, in rugby and other high-contact sports many impacts will be sustained that do not exceed this threshold – these are described as sub-concussive.

Recruiting players of high-contact sport as participants in research exploring the association between mTBI, alcohol use and brain microstructure is advantageous for a relatively homogenous sample and ease of recruitment. In terms of MRI studies, using players of the same sport also has the benefit of minimising the possibility that differences in brain microstructure may be attributable to specialised training in a particular sport. In my study, using only

rugby players had these advantages but was also a limitation because of the potential influence of sub-concussive trauma. In addition, participation in sport has been shown to have an inverse relationship with smoking tobacco and illicit drug use but is related to an increase in alcohol use (Kwan, Bobko, Faulkner, Donnelly, & Cairney, 2014; Lisha & Sussman, 2010). Team sports, rather than individual based sports, are linked with greater alcohol use (Kwan et al., 2014). In future, research using high-contact sport players should also include a group of age-matched controls that play non-contact team sports as a negative exposure control group.

Longitudinal birth cohort studies are excellent resources for continuing to explore the relationship between mTBI, alcohol use and brain structure. The temporal relationship between outcome and exposure can be more easily established in longitudinal research than in cross-sectional research in order to strengthen causal inference. The wealth of information in birth cohort studies make more complex analyses such as dynamic growth analysis possible. One limitation of my research using data from a birth cohort study is the use of self- and/or parent-reported injury, with the majority of incidences based on a single item: "have you/your child had an injury to the head that resulted in a loss of consciousness". Loss of consciousness is a good indicator of TBI as it precludes reporting of an injury to the head below the threshold for TBI, although it is not a necessary symptom for a diagnosis of mTBI. At the same time, while this statement is useful for capturing incidences of mTBI, it does lack specificity. Participants with a reported skull fracture were also included in the mTBI group, this is in keeping with research where ICD codes related to skull fracture were also used to classify mTBI (Winqvist et al., 2007), however, this too lacks

specificity. In future longitudinal studies it would be beneficial to include a comprehensive TBI questionnaire to gather information on the presence or absence of loss of consciousness, post-traumatic amnesia, hospitalisation, cause of injury, post-concussive symptoms and time to resume normal activities. Ideally medical records could be linked in order to corroborate incidences of TBI, however this is quite resource intensive and would not include incidences where the TBI was treated in general practice or where no medical attention was sought. Additionally, studies should continue to include a record of other injuries not involving the head, such as orthopaedic injury, to include a negative control exposure group to increase the ability to infer a causal interpretation.

6.4 IMPLICATIONS AND CONCLUSION

In my thesis, I have found evidence for a causal association between mTBI and risk behaviour, particularly alcohol use. This association should be taken into consideration when treating and managing mTBI in children and adolescents. Currently, 62% of primary and secondary schools in the UK offer counselling services to their pupils for a wide-variety of issues or concerns that affect young people (Department of Education, 2016); this could be a suitable means of support for young people following mTBI. Recovery from brain injury can be a challenging process and even just providing adequate information about mTBI improves recovery trajectories and can alleviate some of the frustration associated with recovery. Counsellors who work in school services should be aware of issues related to mTBI, including increased engagement with risk behaviour, in order to provide necessary support and information.

Additionally, my thesis adds to a growing body of literature linking sport-related mTBI with altered brain microstructure. Sport is one of the leading causes of mTBI in adolescence and young adulthood; in the ALSPAC participants included in Chapter 4, the majority of mTBI incidents reported by participants were sport-related. Playing sport has many benefits for both physical and mental health; however, it is important to also consider the risks of high-contact sports. Recent increased awareness and updates to sport-related mTBI protocols are a positive start and training in this area should continue to be a priority in high-contact sports to protect the players' health.

Overall, my thesis has used a variety of methods to add to the field of mTBI research. Using systematic review and epidemiological analysis of birth cohort data, I have found evidence for an association between mTBI in youth and later risk behaviour, particularly alcohol use. In addition, I have used DTI, an MRI technique that assesses white matter microstructure, to detect differences between participants with and without a recent sport-related mTBI. Future research should continue to use longitudinal data to explore the pathway between mTBI and alcohol use in youth.

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Appendices

Appendix 3.1 Descriptive statistics for participants with injury information included in analyses and participants excluded from analyses due to missing injury information.

	Included *(n=11412)*	**Excluded** *(n=4033)*	p value*
	N (%)	N (%)	
Male	5849 (51.3)	1786 (51.8)	0.553
Social class IV – V [a]	4111 (41.9)	926 (53.6)	<0.001
Rented subsidised housing	1208 (11.4)	731 (25.0)	<0.001
Mother completed secondary school	6496 (62.1)	1587 (78.1)	<0.001
Maternal daily smoking	3034 (28.2)	763 (41.2)	<0.001
Maternal daily alcohol use	1408 (13.1)	103 (5.6)	<0.001
Three or more early life events [b]	5797 (54.4)	330 (27.1)	<0.001
	M (SD)	M (SD)	
Maternal age at birth (years)	28.51 (4.76)	26.16 (5.23)	<0.001
Bonding at 8 months [c]	28.23 (3.65)	28.46 (3.87)	0.040
Positive parenting experience at 21 months [d]	20.78 (2.74)	20.94 (2.90)	0.145
Negative parenting experience at 21 months [d]	5.99 (1.52)	6.00 (1.58)	0.850

Injury from birth to age 16 years (data present n = 11412; data missing n = 4033); * p values calculated using chi square or analysis of variance; [1] highest social class of either parent is skilled non-manual or lower occupation based on the Registrar General's classification of occupations; [b] parent-reported questionnaire relating to upsetting events in the child's life completed when offspring was 6, 30, 42 and 81 months old; [c] parent-report questionnaire completed when offspring was 8 months old; [d] positive and negative parenting experiences based on parent-completed questionnaire when offspring was 21 months old.

Appendix 3.2 Descriptive statistics for covariates on complete case sample for all covariates and all substance use (alcohol, tobacco, cannabis) measures.

	No Injury (n=1,363)	**TBI** (n=207)	**OI** (n=504)	p value*
	N (%)	N (%)	N (%)	
Male	553 (40.6)	112 (54.1)	258 (51.2)	<0.001
Social Class IV – V [a]	450 (33.0)	65 (31.4)	168 (33.3)	0.878
Rented subsidised housing	69 (5.1)	10 (4.8)	18 (3.6)	0.575
Mother completed secondary school	657 (48.2)	93 (44.9)	256 (50.8)	0.338
Maternal daily smoking	241 (17.7)	45 (21.7)	92 (18.3)	0.371
Maternal daily alcohol use	208 (15.3)	36 (17.4)	73 (14.5)	0.619
Three or more early life events [b]	764 (56.0)	132 (63.8)	284 (56.3)	0.120
	M (SD)	M (SD)	M (SD)	
Maternal age at birth (years)	29.84 (4.29)	29.42 (4.26)	29.61 (4.49)	0.600
Bonding at 8 months [c]	27.93 (3.57)	28.06 (3.20)	27.99 (3.49)	0.844
Positive parenting experience at 21 months [d]	5.95 (1.39)	5.94 (1.34)	5.87 (1.34)	0.518
Negative parenting experience at 21 months [d]	20.84 (2.67)	20.55 (2.80)	20.90 (2.58)	0.272

TBI: traumatic brain injury; OI: orthopaedic injury; ***** p values calculated using chi square or analysis of variance; [a] highest social class of either parent is skilled non-manual or lower occupation based on the Registrar General's classification of occupations; [b] parent-reported questionnaire relating to upsetting events in the child's life completed when offspring was 6, 30, 42 and 81 months old; [c] parent-report questionnaire completed when offspring was 8 months old; [d] positive and negative parenting experiences based on parent-completed questionnaire when offspring was 21 months old.

Appendix 3.3 Associations between traumatic brain injury and orthopaedic injuries from birth to age 16 years and substance use at age 17 years on complete case sample

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 2074	n = 2074	n = 2074	n = 2074
TBI vs no Injury	1.54 (1.15 - 2.06)	1.51 (1.13 - 2.03)	1.48 (1.10 - 2.00)	1.31 (0.94 - 1.82)
OI vs no Injury	0.88 (0.72 - 1.09)	0.87 (0.70 - 1.07)	0.87 (0.70 - 1.08)	0.77 (0.61 – 0.98)
TBI vs OI	1.74 (1.26 - 2.41)	1.75 (1.26 - 2.42)	1.71 (1.23 - 2.38)	1.69 (1.17 - 2.45)
Omnibus p	0.541	0.408	0.412	0.080
	Tobacco [b]**			
n	n = 2074	n = 2074	n = 2074	n = 2074
TBI vs no Injury	1.36 (0.98 - 1.89)	1.43 (1.02 - 1.99)	1.37 (0.98 - 1.93)	1.09 (0.74 - 1.62)
OI vs no Injury	1.09 (0.85 - 1.39)	1.12 (0.87 - 1.43)	1.13 (0.88 - 1.46)	1.15 (0.86 - 1.55)
TBI vs OI	1.25 (0.86 - 1.81)	1.27 (0.88 - 1.85)	1.21 (0.83 - 1.77)	0.95 (0.61 - 1.47)
Omnibus p	0.341	0.238	0.227	0.331
	Cannabis [c]**			
n	n = 2074	n = 2074	n = 2074	n = 2074
TBI vs no Injury	1.60 (1.18 - 2.16)	1.56 (1.15 - 2.11)	1.51 (1.11 - 2.05)	1.23 (0.87 - 1.74)
OI vs no Injury	1.10 (0.88 - 1.37)	1.09 (0.87 - 1.37)	1.09 (0.87 - 1.37)	1.02 (0.79 - 1.33)
TBI vs OI	1.46 (1.04 - 2.03)	1.43 (1.02 - 2.00)	1.39 (0.99 - 1.95)	1.20 (0.82 - 1.77)
Omnibus p	0.200	0.236	0.254	0.718

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age and education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.4 Associations between traumatic brain injury from birth to age 16 years, with no additional orthopaedic injury, and substance use at age 17 years compared to orthopaedic injury

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 3564	n = 3148	n = 2778	n = 1992
TBI only vs OI	1.48 (1.10 - 1.99)	1.44 (1.05 - 1.98)	1.57 (1.13 - 2.18)	1.98 (1.28 - 3.09)
Omnibus p	0.056	0.313	0.306	0.081
	Tobacco [b]**			
n	n = 2991	n = 2642	n = 2326	n = 1992
TBI only vs OI	1.14 (0.78 - 1.66)	1.12 (0.75 - 1.68)	1.04 (0.67 - 1.62)	0.96 (0.56 - 1.63)
Omnibus p	0.094	0.072	0.078	0.374
	Cannabis [c]**			
n	n = 3843	n = 3384	n = 2978	n = 1992
TBI only vs OI	1.20 (0.88 - 1.62)	1.09 (0.79 - 1.52)	1.08 (0.76 - 1.53)	1.14 (0.71 - 1.82)
Omnibus p	0.007	0.078	0.109	0.588

Sample size reduces per adjustment as the participants who are missing covariate data get excluded TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis

Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.5 Associations between traumatic brain injury from birth to age 16 years, with no additional orthopaedic injury, and substance use at age 17 years compared to orthopaedic injury on complete case sample

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 1992	n = 1992	n = 1992	n = 1992
TBI only vs OI	1.81 (1.22 - 2.68)	1.82 (1.22 - 2.70)	1.81 (1.22 - 2.70)	1.98 (1.28 - 3.09)
Omnibus p	0.435	0.328	0.344	0.081
	Tobacco [b]**			
n	n = 1992	n = 1992	n = 1992	n = 1992
TBI only vs OI	1.08 (0.68 - 1.71)	1.12 (0.71 - 1.78)	1.10 (0.69 - 1.76)	0.96 (0.56 - 1.63)
Omnibus p	0.446	0.324	0.302	0.374
	Cannabis [c]**			
n	n = 1992	n = 1992	n = 1992	n = 1992
TBI only vs OI	1.24 (0.82 - 1.87)	1.23 (0.81 - 1.86)	1.22 (0.80 - 1.85)	1.14 (0.71 - 1.82)
Omnibus p	0.319	0.355	0.365	0.588

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.6 Associations between traumatic brain injury and orthopaedic injuries from birth to age 16 years and criminal behaviours at age 17 years on complete case sample

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 2115	n = 2115	n = 2115	n = 2115
TBI vs no Injury	1.82 (1.30 - 2.54)	1.62 (1.15 - 2.28)	1.58 (1.13 - 2.23)	1.29 (0.09 - 1.88)
OI vs no Injury	1.71 (1.34 - 2.18)	1.57 (1.22 - 2.01)	1.56 (1.22 - 2.00)	1.67 (1.27 - 2.19)
TBI vs OI	1.06 (0.74 - 1.53)	1.03 (0.72 - 1.49)	1.01 (0.70 - 1.47)	0.77 (0.52 - 1.16)
Omnibus p	<0.001	<0.001	<0.001	<0.001
	Trouble with the Police [b]**			
n	n = 2077	n = 2077	n = 2077	n = 2077
TBI vs no Injury	1.73 (1.20 – 2.48)	1.52 (1.05 – 2.21)	1.49 (1.02 – 2.17)	1.17 (0.77 – 1.77)
OI vs no Injury	1.15 (0.87 – 1.53)	1.01 (0.75 – 1.35)	1.02 (0.76 – 1.36)	1.03 (0.75 – 1.42)
TBI vs OI	1.50 (1.00 - 2.25)	1.51 (1.00 - 2.30)	1.46 (0.96 - 2.23)	1.14 (0.71 - 1.81)
Omnibus p	0.158	0.707	0.678	0.765

Complete cases had no missing data for the exposure, outcome or covariates TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.7 Associations between traumatic brain injury from birth to age 16 years, with no additional orthopaedic injury, and criminal behaviour at age 17 years compared to orthopaedic injury

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 3719	n = 3283	n = 2886	n = 2031
TBI only vs OI	1.14 (0.81 - 1.60)	1.15 (0.80 - 1.66)	1.18 (0.81 - 1.73)	0.76 (0.46 - 1.26)
Omnibus p	<0.001	0.001	0.001	<0.001
	Trouble with the Police [b]**			
n	n = 3657	n = 3228	n = 2844	n = 1995
TBI only vs OI	0.94 (0.63 - 1.40)	0.86 (0.55 - 1.34)	0.90 (0.57 - 1.44)	0.80 (0.44 - 1.47)
Omnibus p	0.001	0.096	0.102	0.930

Sample size reduces per adjustment as the participants who are missing covariate data get excluded TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.8 Associations between traumatic brain injury from birth to age 16 years, with no additional orthopaedic injury, and criminal behaviour at age 17 years compared to orthopaedic injury on complete case sample

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 2031	n = 2031	n = 2031	n = 2031
TBI only vs OI	0.94 (0.60 - 1.46)	0.95 (0.60 - 1.49)	0.95 (0.60 - 1.50)	0.76 (0.46 - 1.26)
Omnibus p	<0.001	<0.001	<0.001	<0.001
	Trouble with the Police [b]**			
n	n = 1995	n = 1995	n = 1995	n = 1995
TBI only vs OI	1.02 (0.60 - 1.74)	1.03 (0.60 - 1.79)	1.02 (0.59 - 1.76)	0.80 (0.44 - 1.47)
Omnibus p	0.299	0.956	0.921	0.930

Complete cases had no missing data for the exposure, outcome or covariates TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.9 Associations between traumatic brain injury and orthopaedic injuries from birth to age 16 years and psychiatric symptoms based on the Strengths and Difficulties Questionnaire at age 17 years on complete case sample

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 4493	n = 4493	n = 4493
TBI vs no Injury	1.64 (1.11 – 2.43)	1.72 (1.16 – 2.55)	1.62 (1.08 – 2.41)
OI vs no Injury	1.08 (0.79 – 1.48)	1.10 (0.80 – 1.50)	1.07 (0.78 – 1.47)
TBI vs OI	1.52 (0.97 - 2.36)	1.57 (1.01 - 2.45)	1.51 (0.96 - 2.37)
Omnibus p	0.391	0.340	0.445
	Peer Problems [b]*		
n	n = 4483	n = 4483	n = 4483
TBI vs no Injury	0.92 (0.62 – 1.37)	0.88 (0.59 – 1.31)	0.85 (0.57 – 1.26)
OI vs no Injury	0.84 (0.64 – 1.11)	0.81 (0.61 – 1.07)	0.79 (0.60 – 1.05)
TBI vs OI	1.10 (0.71 - 1.71)	1.09 (0.70 - 1.69)	1.07 (0.68 - 1.67)
Omnibus p	0.206	0.127	0.090

Complete cases had no missing data for the exposure, outcome or covariates TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.10 Associations between traumatic brain injury from birth to age 16 years, with no additional orthopaedic injury, and psychiatric symptoms at age 17 years compared to orthopaedic injury

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 2437	n = 4818	n = 4328
TBI only vs OI	1.51 (0.96 - 2.38)	1.74 (1.07 - 2.84)	1.80 (1.08 - 3.00)
Omnibus p	0.201	0.285	0.458
	Peer Problems [b]*		
n	n = 5427	n = 4806	n = 4316
TBI only vs OI	1.17 (0.75 - 1.83)	1.17 (0.71 - 1.93)	1.15 (0.68 - 1.96)
Omnibus p	0.829	0.121	0.100

Sample size reduces per adjustment as the participants who are missing covariate data get excluded TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.11 Associations between traumatic brain injury from birth to age 16 years, with no additional orthopaedic injury, and psychiatric symptoms at age 17 years compared to orthopaedic injury on complete case sample

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 4328	n = 4328	n = 4328
TBI only vs OI	1.76 (1.06 - 2.91)	1.85 (1.12 - 3.07)	1.80 (1.08 - 3.00)
Omnibus p	0.412	0.366	0.458
	Peer Problems [b]*		
n	n = 4316	n = 4316	n = 4316
TBI only vs OI	1.15 (0.68 - 1.95)	1.16 (0.68 - 1.96)	1.15 (0.68 - 1.96)
Omnibus p	0.217	0.133	0.100

Complete cases had no missing data for the exposure, outcome or covariates TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.12 Association between traumatic brain injury and orthopaedic injuries from birth to age 11 years and substance use at age 17 years

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 3188	n = 2812	n = 2381	n = 1788
TBI vs no Injury	1.26 (0.89 - 1.78)	1.28 (0.89 - 1.85)	1.37 (0.93 – 2.02)	1.13 (0.68 - 1.88)
OI vs no Injury	1.16 (0.97 - 1.38)	1.10 (0.91 – 1.33)	1.13 (0.92 - 1.38)	0.81 (0.61 - 1.07)
TBI vs OI	1.09 (0.75 - 1.57)	1.16 (0.78 - 1.72)	1.22 (0.81 - 1.85)	1.40 (0.80 - 2.44)
Omnibus p	0.072	0.231	0.266	0.167
	Tobacco [b]**			
n	n = 2675	n = 2364	n = 2084	n = 1788
TBI vs no Injury	1.35 (0.88 – 2.08)	1.31 (0.83 – 2.07)	1.15 (0.69 – 1.92)	1.00 (0.53 - 1.87)
OI vs no Injury	1.01 (0.80 – 1.28)	1.02 (0.79 – 1.32)	1.00 (0.76 – 1.32)	0.83 (0.58 - 1.19)
TBI vs OI	1.34 (0.84 - 2.15)	1.28 (0.78 - 2.12)	1.16 (0.66 - 2.01)	1.20 (0.60 - 2.39)
Omnibus p	0.786	0.739	0.940	0.328
	Cannabis [c]**			
n	n = 3436	n = 3023	n = 2668	n = 1788
TBI vs no Injury	1.61 (1.14 - 2.28)	1.44 (0.99 – 2.08)	1.45 (0.98 - 2.15)	1.47 (0.88 - 2.47)
OI vs no Injury	1.17 (0.98 - 1.41)	1.12 (0.92 – 1.37)	1.10 (0.89 - 1.36)	1.03 (0.76 - 1.40)
TBI vs OI	1.38 (0.95 - 2.00)	1.28 (0.86 - 1.91)	1.32 (0.86 - 2.02)	1.43 (0.81 - 2.52)
Omnibus p	0.041	0.162	0.254	0.671

Sample size reduces per adjustment as the participants who are missing covariate data get excluded TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test

(AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.13 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and substance use at age 17 years compared to orthopaedic injury

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 3144	n = 2776	n = 2454	n = 1762
TBI only vs OI	1.37 (0.89 - 2.11)	1.40 (0.89 - 2.20)	1.47 (0.91 - 2.37)	1.64 (0.87 - 3.13)
Omnibus p	0.059	0.207	0.243	0.181
	Tobacco [b]**			
n	n = 2640	n = 2333	n = 2055	n = 1762
TBI only vs OI	1.30 (0.74 - 2.27)	1.31 (0.73 - 2.35)	1.16 (0.61 - 2.21)	0.98 (0.43 - 2.20)
Omnibus p	0.851	0.774	0.968	0.275
	Cannabis [c]**			
n	n = 3391	n = 2986	n = 2635	n = 1762
TBI only vs OI	1.53 (1.00 - 2.36)	1.43 (0.91 - 2.25)	1.47 (0.91 - 2.37)	1.51 (0.79 - 2.87)
Omnibus p	0.044	0.164	0.259	0.214

Sample size reduces per adjustment as the participants who are missing covariate data get excluded TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.14 Associations between traumatic brain injury and orthopaedic injuries from birth to age 11 years and criminal behaviour at age 17 years

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 3325	n = 2931	n = 2584	n = 1818
TBI vs no Injury	1.26 (0.82 - 1.94)	1.15 (0.72 - 1.83)	1.25 (0.77 - 2.02)	0.97 (0.53 - 1.79)
OI vs no Injury	1.30 (1.05 - 1.62)	1.31 (1.03 - 1.66)	1.37 (1.06 - 1.75)	1.78 (1.30 - 2.45)
TBI vs OI	0.97 (0.61 - 1.53)	0.88 (0.53 - 1.44)	0.91 (0.54 - 1.53)	0.54 (0.28 - 1.04)
Omnibus p	0.013	0.025	0.012	0.001
	Trouble with the Police [b]**			
n	n = 3275	n = 2886	n = 2549	n = 1790
TBI vs no Injury	1.44 (0.90 – 2.28)	1.20 (0.73 – 1.98)	1.31 (0.78 - 2.21)	1.17 (0.62 - 2.22)
OI vs no Injury	1.34 (1.05 – 1.70)	1.28 (0.99 - 1.67)	1.27 (0.96 - 1.69)	1.15 (0.79 - 1.69)
TBI vs OI	1.06 (0.66 - 1.76)	0.94 (0.55 - 1.60)	1.03 (0.59 - 1.81)	1.02 (0.50 - 2.06)
Omnibus p	0.012	0.056	0.078	0.432

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.15 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and criminal behaviour at age 17 years compared to orthopaedic injury

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 3285	n = 2898	n = 2555	n = 1792
TBI only vs OI	1.08 (0.64 - 1.83)	1.00 (0.58 - 1.75)	1.07 (0.60 - 1.91)	0.65 (0.31 - 1.34)
Omnibus p	0.012	0.021	0.010	<0.001
	Trouble with the Police [b]**			
n	n = 3235	n = 2852	n = 2519	n = 1764
TBI only vs OI	1.14 (0.65 - 2.00)	0.98 (0.53 - 1.79)	1.08 (0.57 - 2.05)	1.04 (0.47 - 2.29)
Omnibus p	0.012	0.055	0.080	0.434

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.16 Associations between traumatic brain injury and orthopaedic injuries from birth to age 11 years and psychiatric symptoms based on the Strengths and Difficulties Questionnaire at age 17 years

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 4923	n = 4372	n = 3937
TBI vs no Injury	2.20 (1.37 – 3.53)	2.33 (1.41 – 3.85)	1.90 (1.11 - 3.26)
OI vs no Injury	1.05 (0.76 – 1.44)	0.99 (0.69 – 1.41)	0.96 (0.66 - 1.39)
TBI vs OI	2.10 (1.23 - 3.57)	2.35 (1.33 - 4.17)	1.98 (1.08 - 3.65)
Omnibus p	0.442	0.656	0.884
	Peer Problems [b]*		
n	n = 4912	n = 4359	n = 3924
TBI vs no Injury	1.51 (0.95 – 2.39)	1.30 (0.79 – 2.13)	0.99 (0.57 - 1.21)
OI vs no Injury	1.01 (0.77 – 1.31)	0.91 (0.68 – 1.22)	0.89 (0.65 - 1.21)
TBI vs OI	1.51 (0.91 - 2.49)	1.42 (0.83 - 2.45)	1.12 (0.61 - 2.05)
Omnibus p	0.780	0.666	0.456

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.17 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and psychiatric symptoms at age 17 years compared to orthopaedic injury

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 4854	n = 4310	n = 3880
TBI only vs OI	2.65 (1.48 - 4.74)	3.04 (1.64 - 5.64)	2.77 (1.45 - 5.31)
Omnibus p	0.450	0.651	0.826
	Peer Problems [b]*		
n	n = 4842	n = 4296	n = 3866
TBI only vs OI	1.58 (0.88 - 2.84)	1.47 (0.78 - 2.77)	1.28 (0.64 - 2.54)
Omnibus p	0.828	0.639	0.481

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.18 Associations between traumatic brain injury and orthopaedic injuries from birth to age 11 years and psychiatric symptoms based on the Development and Well-Being Assessment (DAWBA) at age 15 years

DAWBA	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Externalising Behaviour [a]*		
n	n = 3994	n = 3515	n = 3112
TBI vs no Injury	2.25 (1.32 – 3.81)	2.35 (1.35 – 4.11)	1.83 (0.98 - 3.41)
OI vs no Injury	0.85 (0.57 – 1.27)	0.90 (0.59 – 1.39)	0.87 (0.55 - 1.38)
TBI vs OI	2.65 (1.43 - 4.91)	2.61 (1.36 - 4.98)	2.11 (1.03 - 4.32)
Omnibus p	0.779	0.970	0.796
	ODD [b]**		
n	n = 3983	n = 3506	n = 3105
TBI vs no Injury	2.28 (1.28 – 4.07)	2.42 (1.31 – 4.55)	1.78 (0.89 - 3.58)
OI vs no Injury	0.81 (0.51 – 1.28)	0.98 (0.61 – 1.56)	0.98 (0.60 - 1.61)
TBI vs OI	2.82 (1.42 - 5.59)	2.48 (1.22 - 5.02)	1.82 (0.82 - 4.01)
Omnibus p	0.673	0.730	0.851
	CD [c]**		
n	n = 3982	n = 3505	n = 3104
TBI vs no Injury	1.09 (0.34 – 3.55)	0.83 (0.20 – 3.53)	0.72 (0.17 - 3.10)
OI vs no Injury	0.66 (0.31 - 1.40)	0.78 (0.36 – 1.70)	0.85 (0.39 - 1.86)
TBI vs OI	1.66 (0.44 - 6.33)	1.06 (0.22 - 5.10)	0.94 (0.17 - 4.13)
Omnibus p	0.301	0.523	0.636
	ADHD [d]**		

n	n = 3994	n = 3515	n = 3112
TBI vs no Injury	3.15 (1.07 – 9.28)	2.86 (0.94 – 8.70)	3.02 (0.97 - 9.36)
OI vs no Injury	1.42 (0.62 - 3.21)	0.91 (0.34 – 2.47)	0.57 (0.16 - 1.98)
TBI vs OI	2.23 (0.66 - 7.48)	3.14 (0.82 - 12.02)	5.28 (1.14 - 24.51)
Omnibus p	0.274	0.891	0.623

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; * logistic regression; [a] externalising disorder symptoms based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [b] ODD: oppositional defiant disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [c] CD: conduct disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [d] ADHD: attentional defiant hyperactivity disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.19 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and psychiatric symptoms based on the Development and Well-Being Assessment (DAWBA) at age 15 years compared to orthopaedic injury

DAWBA	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Externalising Behaviour [a]*		
n	n = 3931	n = 3460	n = 3063
TBI only vs OI	2.34 (1.11 - 4.92)	2.20 (1.00 - 4.83)	1.99 (0.85 - 4.64)
Omnibus p	0.609	0.833	0.698
	ODD [b]**		
n	n = 3920	n = 3451	n = 3056
TBI only vs OI	2.76 (1.25 - 6.12)	2.29 (0.99 - 5.28)	1.94 (0.79 - 4.79)
Omnibus p	0.553	0.876	0.901
	CD [c]**		
n	n = 3919	n = 3450	n = 3055
TBI only vs OI	1.69 (0.35 - 8.06)	0.83 (0.10 - 6.77)	0.67 (0.08 - 5.60)
Omnibus p	0.294	0.512	0.652
	ADHD		
n	n = 3931	n = 3460	n = 3063
TBI only vs OI	1.68 (0.35 - 8.03)	2.53 (0.48 - 13.39)	4.03 (0.65 - 25.14)
Omnibus p	0.345	0.990	0.514

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; * logistic regression; [a] externalising disorder symptoms based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [b] ODD: oppositional defiant disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [c] CD: conduct disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15

years; [d] ADHD: attentional defiant hyperactivity disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.20 Association between traumatic brain injury and orthopaedic injuries from birth to age 11 years and substance use at age 17 years on complete case sample

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 1788	n = 1788	n = 1788	n = 1788
TBI vs no Injury	1.25 (0.80 - 1.96)	1.24 (0.79 - 1.94)	1.18 (0.75 - 1.86)	1.13 (0.68 - 1.88)
OI vs no Injury	0.88 (0.69 - 1.13)	0.88 (0.69 - 1.12)	0.87 (0.68 - 1.12)	0.81 (0.61 - 1.07)
TBI vs OI	1.42 (0.87 - 2.31)	1.41 (0.86 - 2.30)	1.35 (0.82 - 2.21)	1.40 (0.80 - 2.44)
Omnibus p	0.429	0.388	0.360	0.167
	Tobacco [b]**			
n	n = 1788	n = 1788	n = 1788	n = 1788
TBI vs no Injury	1.09 (0.64 - 1.87)	1.08 (0.63 - 1.85)	0.99 (0.57 - 1.72)	1.00 (0.53 - 1.87)
OI vs no Injury	0.90 (0.66 - 1.21)	0.91 (0.68 - 1.24)	0.91 (0.67 - 1.23)	0.83 (0.58 - 1.19)
TBI vs OI	1.22 (0.68 - 2.19)	1.18 (0.65 - 2.13)	1.09 (0.60 - 2.00)	1.20 (0.60 - 2.39)
Omnibus p	0.518	0.596	0.532	0.328
	Cannabis [c]**			
n	n = 1788	n = 1788	n = 1788	n = 1788
TBI vs no Injury	1.45 (0.92 - 2.29)	1.47 (0.92 - 2.32)	1.37 (0.86 - 2.18)	1.47 (0.88 - 2.47)
OI vs no Injury	1.03 (0.79 - 1.33)	1.05 (0.80 - 1.36)	1.04 (0.80 - 1.36)	1.03 (0.76 - 1.40)
TBI vs OI	1.41 (0.86 - 2.33)	1.40 (0.85 - 2.32)	1.32 (0.79 - 2.19)	1.43 (0.81 - 2.52)
Omnibus p	0.659	0.557	0.615	0.671

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.21 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and substance use at age 17 years compared to orthopaedic injury on complete case sample

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 1762	n = 1762	n = 1762	n = 1762
TBI only vs OI	1.72 (0.98 - 3.04)	1.70 (0.96 - 3.02)	1.61 (0.91 - 2.87)	1.64 (0.87 - 3.13)
Omnibus p	0.453	0.412	0.383	0.181
	Tobacco [b]**			
n	n = 1762	n = 1762	n = 1762	n = 1762
TBI only vs OI	1.18 (0.59 - 2.35)	1.17 (0.58 - 2.34)	1.07 (0.53 - 2.17)	0.98 (0.43 - 2.20)
Omnibus p	0.495	0.577	0.511	0.275
	Cannabis [c]**			
n	n = 1762	n = 1762	n = 1762	n = 1762
TBI only vs OI	1.58 (0.89 - 2.81)	1.58 (0.88 - 2.81)	1.47 (0.82 - 2.63)	1.51 (0.79 - 2.87)
Omnibus p	0.672	0.568	0.622	0.214

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.22 Associations between traumatic brain injury and orthopaedic injuries from birth to age 11 years and criminal behaviour at age 17 years on complete case sample

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 1818	n = 1818	n = 1818	n = 1818
TBI vs no Injury	1.28 (0.74 - 2.21)	1.12 (0.64 - 1.96)	1.06 (0.60 - 1.86)	0.97 (0.53 - 1.79)
OI vs no Injury	1.57 (1.18 - 2.08)	1.57 (1.18 - 2.10)	1.57 (1.17 - 2.09)	1.78 (1.30 - 2.45)
TBI vs OI	0.82 (0.46 - 1.46)	0.71 (0.39 - 1.29)	0.68 (0.37 - 1.23)	0.54 (0.28 - 1.04)
Omnibus p	0.002	0.002	0.003	0.001
	Trouble with the Police [b]**			
n	n = 1790	n = 1790	n = 1790	n = 1790
TBI vs no Injury	1.50 (0.85 - 2.63)	1.24 (0.69 - 2.22)	1.17 (0.65 - 2.11)	1.17 (0.62 - 2.22)
OI vs no Injury	1.11 (0.79 - 1.54)	1.06 (0.75 - 1.50)	1.07 (0.76 - 1.51)	1.15 (0.79 - 1.69)
TBI vs OI	1.35 (0.73 - 2.51)	1.16 (0.61 - 2.21)	1.09 (0.57 - 2.08)	1.02 (0.50 - 2.06)
Omnibus p	0.422	0.654	0.635	0.432

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.23 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and criminal behaviour at age 17 years compared to orthopaedic injury on complete case sample

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 1792	n = 1792	n = 1792	n = 1792
TBI only vs OI	0.95 (0.49 - 1.84)	0.83 (0.42 - 1.62)	0.80 (0.40 - 1.57)	0.65 (0.31 - 1.34)
Omnibus p	0.001	0.002	0.002	<0.001
	Trouble with the Police [b]**			
n	n = 1764	n = 1764	n = 1764	n = 1764
TBI only vs OI	1.48 (0.74 - 2.99)	1.25 (0.61 - 2.58)	1.16 (0.56 - 2.42)	1.04 (0.47 - 2.29)
Omnibus p	0.431	0.650	0.634	0.434

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.24 Associations between traumatic brain injury and orthopaedic injuries from birth to age 11 years and psychiatric symptoms based on the Strengths and Difficulties Questionnaire at age 17 years on complete case sample

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 3937	n = 3937	n = 3937
TBI vs no Injury	2.14 (1.26 – 3.63)	2.17 (1.28 - 3.70)	1.90 (1.11 - 3.26)
OI vs no Injury	0.98 (0.68 – 1.41)	0.99 (0.68 – 1.43)	0.96 (0.66 - 1.39)
TBI vs OI	2.19 (1.20 - 3.98)	2.20 (1.21 - 4.01)	1.98 (1.08 - 3.65)
Omnibus p	0.752	0.708	0.884
	Peer Problems [b]*		
n	n = 3924	n = 3924	n = 3924
TBI vs no Injury	1.17 (0.68 - 2.02)	1.10 (0.63 - 1.90)	0.99 (0.57 - 1.21)
OI vs no Injury	0.92 (0.68 - 1.25)	0.91 (0.67 - 1.24)	0.89 (0.65 - 1.21)
TBI vs OI	1.26 (0.70 - 2.29)	1.20 (0.66 - 2.19)	1.12 (0.61 - 2.05)
Omnibus p	0.684	0.605	0.456

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.25 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and psychiatric symptoms at age 17 years compared to orthopaedic injury on complete case sample

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 3880	n = 3880	n = 3880
TBI only vs OI	3.02 (1.60 - 5.69)	3.05 (1.61 - 5.76)	2.77 (1.45 - 5.31)
Omnibus p	0.716	0.667	0.826
	Peer Problems [b]*		
n	n = 3866	n = 3866	n = 3866
TBI only vs OI	1.42 (0.72 - 2.80)	1.36 (0.69 - 2.69)	1.28 (0.64 - 2.54)
Omnibus p	0.695	0.623	0.481

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.26 Associations between traumatic brain injury and orthopaedic injuries from birth to age 11 years and psychiatric symptoms based on the Development and Well-Being Assessment (DAWBA) at age 15 years on complete case sample

DAWBA	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Externalising Behaviour [a]*		
n	n = 3112	n = 3112	n = 3112
TBI vs no Injury	2.19 (1.20 - 4.00)	2.11 (1.15 - 3.88)	1.83 (0.98 - 3.41)
OI vs no Injury	0.88 (0.56 - 1.39)	0.88 (0.55 - 1.38)	0.87 (0.55 - 1.38)
TBI vs OI	2.49 (1.24 - 5.01)	2.41 (1.19 - 4.87)	2.11 (1.03 - 4.32)
Omnibus p	0.894	0.866	0.796
	ODD [b]**		
n	n = 3105	n = 3105	n = 3105
TBI vs no Injury	2.11 (1.07 - 4.17)	2.04 (1.03 - 4.04)	1.78 (0.89 - 3.58)
OI vs no Injury	0.98 (0.60 - 1.60)	0.98 (0.60 - 1.60)	0.98 (0.60 - 1.61)
TBI vs OI	2.15 (0.99 - 4.67)	2.08 (0.96 - 4.54)	1.82 (0.82 - 4.01)
Omnibus p	0.797	0.820	0.851
	CD [c]**		
n	n = 3104	n = 3104	n = 3104
TBI vs no Injury	0.91 (0.22 – 3.82)	0.90 (0.21 – 3.80)	0.72 (0.17 - 3.10)
OI vs no Injury	0.83 (0.38 - 1.81)	0.83 (0.38 - 1.81)	0.85 (0.39 - 1.86)
TBI vs OI	1.09 (0.23 - 5.19)	1.08 (0.23 - 5.17)	0.94 (0.17 - 4.13)
Omnibus p	0.641	0.636	0.636
	ADHD [d]**		

n	n = 3112	n = 3112	n = 3112
TBI vs no Injury	3.69 (1.23 - 11.12)	3.39 (1.11 - 10.37)	3.02 (0.97 - 9.36)
OI vs no Injury	0.62 (0.18 - 2.13)	0.60 (0.18 - 2.07)	0.57 (0.16 - 1.98)
TBI vs OI	5.94 (1.31 - 26.81)	5.62 (1.23 - 25.65)	5.28 (1.14 - 24.51)
Omnibus p	0.758	0.702	0.623

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; * logistic regression; [a] externalising disorder symptoms based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [b] ODD: oppositional defiant disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [c] CD: conduct disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [d] ADHD: attentional defiant hyperactivity disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.27 Associations between traumatic brain injury from birth to age 11 years, with no additional orthopaedic injury, and psychiatric symptoms based on the Development and Well-Being Assessment (DAWBA) at age 15 years compared to orthopaedic injury on complete case sample

DAWBA	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Externalising Behaviour [a]*		
n	n = 3063	n = 3063	n = 3063
TBI only vs OI	2.25 (0.98 - 5.17)	2.31 (1.01 - 5.32)	1.99 (0.85 - 4.64)
Omnibus p	0.759	0.747	0.698
	ODD [b]**		
n	n = 3056	n = 3056	n = 3056
TBI only vs OI	2.25 (0.93 - 5.44)	2.28 (0.94 - 5.52)	1.94 (0.79 - 4.79)
Omnibus p	0.863	0.880	0.901
	CD [c]**		
n	n = 3055	n = 3055	n = 3055
TBI only vs OI	0.81 (0.10 - 6.54)	0.88 (0.11 - 7.12)	0.67 (0.08 - 5.60)
Omnibus p	0.617	0.624	0.652
	ADHD		
n	n = 3063	n = 3063	n = 3063
TBI only vs OI	4.38 (0.72 - 26.52)	4.77 (0.78 - 29.23)	4.03 (0.65 - 25.14)
Omnibus p	0.585	0.565	0.514

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; * logistic regression; [a] externalising disorder symptoms based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [b] ODD: oppositional defiant disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [c] CD:

conduct disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years; [d] ADHD: attentional defiant hyperactivity disorder based on the Development and Well-being Assessment (DAWBA) self-reported at age 15 years

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.28 Associations between traumatic brain injury and orthopaedic injuries from age 12 to age 16 years and substance use at 17 years

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 2926	n = 2580	n = 2263	n = 1649
TBI vs no Injury	1.71 (1.28 - 2.27)	1.59 (1.17 - 2.15)	1.72 (1.25 - 2.37)	1.41 (0.93 - 2.15)
OI vs no Injury	1.06 (0.83 - 1.35)	0.98 (0.75 - 1.27)	0.98 (0.74 - 1.31)	0.70 (0.47 - 1.04)
TBI vs OI	1.61 (1.13 - 2.31)	1.62 (1.11 - 2.38)	1.76 (1.17 - 2.63)	2.03 (1.17 - 3.53)
Omnibus p	0.116	0.432	0.319	0.282
	Tobacco [b]**			
n	n = 2488	n = 2193	n = 1923	n = 1649
TBI vs no Injury	1.56 (1.11 – 2.19)	1.67 (1.16 – 2.41)	1.71 (1.15 - 2.52)	1.15 (0.72 - 1.86)
OI vs no Injury	1.50 (1.13 - 2.00)	1.61 (1.17 - 2.21)	1.76 (1.25 - 2.48)	2.00 (1.29 - 3.09)
TBI vs OI	1.04 (0.68 - 1.58)	1.04 (0.66 - 1.63)	0.97 (0.60 - 1.57)	0.58 (0.32 - 1.06)
Omnibus p	0.001	0.000	0.000	0.003
	Cannabis [c]**			
n	n = 3172	n = 2788	n = 2439	n = 1649
TBI vs no Injury	1.49 (1.11 - 1.99)	1.32 (0.97 - 1.81)	1.36 (0.98 - 1.88)	1.14 (0.74 - 1.76)
OI vs no Injury	1.32 (1.03 - 1.68)	1.23 (0.95 - 1.60)	1.28 (0.96 - 1.72)	1.04 (0.69 - 1.58)
TBI vs OI	1.13 (0.79 - 1.62)	1.08 (0.73 - 1.58)	1.06 (0.70 - 1.60)	1.09 (0.62 - 1.92)
Omnibus p	0.004	0.051	0.034	0.702

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test

(AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.29 Associations between traumatic brain injury from age 12 to age 16 years, with no additional orthopaedic injury, and substance use at age 17 years compared to orthopaedic injury

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 2890	n = 2548	n = 2232	n = 1625
TBI only vs OI	1.59 (1.09 - 2.32)	1.58 (1.06 - 2.37)	1.76 (1.14 - 2.71)	2.41 (1.35 - 4.29)
Omnibus p	0.172	0.540	0.403	0.364
	Tobacco [b]**			
n	n = 2455	n = 2164	n = 1895	n = 1625
TBI only vs OI	0.95 (0.60 - 1.49)	0.89 (0.54 - 1.47)	0.82 (0.48 - 1.40)	0.59 (0.31 - 1.13)
Omnibus p	0.002	0.001	<0.001	0.002
	Cannabis [c]**			
n	n = 3135	n = 2755	n = 2407	n = 1625
TBI only vs OI	1.06 (0.72 - 1.56)	0.997 (0.66 - 1.51)	0.97 (0.62 - 1.51)	1.12 (0.61 - 2.03)
Omnibus p	0.008	0.070	0.051	0.714

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.30 Associations between traumatic brain injury and orthopaedic injuries from age 12 to 16 years and criminal behaviour at age 17 years

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 3079	n = 2718	n = 2372	n = 1681
TBI vs no Injury	2.05 (1.50 - 2.80)	1.88 (1.34 - 2.63)	1.99 (1.40 - 2.82)	1.52 (0.97 - 2.39)
OI vs no Injury	1.89 (1.44 - 2.45)	1.47 (1.09 - 1.97)	1.53 (1.11 - 2.11)	1.49 (0.99 - 2.24)
TBI vs OI	1.09 (0.74 - 1.60)	1.28 (0.85 - 1.94)	1.30 (0.83 - 2.01)	1.02 (0.58 - 1.79)
Omnibus p	<0.001	0.001	<0.001	0.022
	Trouble with the Police [b]**			
n	n = 3024	n = 2668	n = 2339	n = 1651
TBI vs no Injury	1.74 (1.22 – 2.48)	1.43 (0.96 - 2.13)	1.51 (1.00 - 2.29)	1.21 (0.72 - 2.03)
OI vs no Injury	1.59 (1.17 - 2.17)	1.09 (0.77 - 1.54)	1.12 (0.77 - 1.64)	0.86 (0.52 - 1.42)
TBI vs OI	1.09 (0.70 - 1.70)	1.31 (0.80 - 2.15)	1.35 (0.80 - 2.27)	1.41 (0.72 - 2.77)
Omnibus p	<0.001	0.360	0.252	0.756

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.31 Associations between traumatic brain injury from age 12 to age 16 years, with no additional orthopaedic injury, and criminal behaviour at age 17 years compared to orthopaedic injury

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 3043	n = 2682	n = 2341	n = 1657
TBI only vs OI	0.94 (0.62 - 1.42)	1.09 (0.70 - 1.71)	1.09 (0.67 - 1.75)	0.85 (0.46 - 1.58)
Omnibus p	<0.001	0.002	0.002	0.041
	Trouble with the Police [b]**			
n	n = 2988	n = 2636	n = 2308	n = 1627
TBI only vs OI	0.80 (0.48 - 1.31)	0.90 (0.51 - 1.58)	0.90 (0.50 - 1.64)	0.92 (0.43 - 1.99)
Omnibus p	0.002	0.662	0.538	0.435

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.32 Associations between traumatic brain injury and orthopaedic injuries from age 12 to 16 years and psychiatric symptoms based on the Strengths and Difficulties Questionnaire at age 17 years

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 4460	n = 3939	n = 3536
TBI vs no Injury	1.21 (0.74 – 1.96)	1.41 (0.84 – 2.38)	1.39 (0.81 - 2.39)
OI vs no Injury	1.39 (0.92 - 2.12)	1.46 (0.92 - 2.31)	1.37 (0.84 - 2.24)
TBI vs OI	0.87 (0.47 - 1.59)	0.97 (0.50 - 1.85)	1.02 (0.51 - 2.02)
Omnibus p	0.095	0.058	0.126
	Peer Problems [b]*		
n	n = 4452	n = 3929	n = 3526
TBI vs no Injury	0.87 (0.55 – 1.38)	0.75 (0.45 – 1.25)	0.72 (0.42 - 1.24)
OI vs no Injury	0.87 (0.57 - 1.32)	0.56 (0.34 - 0.93)	0.55 (0.32 - 0.95)
TBI vs OI	1.00 (0.55 - 1.81)	1.35 (0.67 - 2.69)	1.30 (0.62 - 2.74)
Omnibus p	0.421	0.014	0.018

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.33 Associations between traumatic brain injury from age 12 to age 16 years, with no additional orthopaedic injury, and psychiatric symptoms at age 17 years compared to orthopaedic injury

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 4404	n = 3890	n = 3490
TBI only vs OI	1.01 (0.54 - 1.87)	1.13 (0.58 - 2.19)	1.18 (0.58 - 2.38)
Omnibus p	0.065	0.042	0.096
	Peer Problems [b]*		
n	n = 4396	n = 3880	n = 3480
TBI only vs OI	1.17 (0.64 - 2.14)	1.58 (0.78 - 3.21)	1.51 (0.71 - 3.22)
Omnibus p	0.560	0.024	0.029

Sample size reduces per adjustment as the participants who are missing covariate data get excluded. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.34 Associations between traumatic brain injury and orthopaedic injuries from age 12 to age 16 years and substance use at 17 years on complete case sample

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 1649	n = 1649	n = 1649	n = 1649
TBI vs no Injury	1.76 (1.22 - 2.54)	1.72 (1.19 - 2.49)	1.71 (0.60 - 1.20)	1.41 (0.93 - 2.15)
OI vs no Injury	0.88 (0.63 - 1.24)	0.84 (0.59 - 1.18)	0.85 (0.60 - 1.20)	0.70 (0.47 - 1.04)
TBI vs OI	2.00 (1.24 - 3.21)	2.05 (1.27 - 3.31)	2.02 (1.24 - 3.27)	2.03 (1.17 - 3.53)
Omnibus p	0.721	0.964	0.915	0.282
	Tobacco [b]**			
n	n = 1649	n = 1649	n = 1649	n = 1649
TBI vs no Injury	1.55 (1.04 - 2.31)	1.70 (1.13 - 2.55)	1.69 (1.12 - 2.55)	1.15 (0.72 - 1.86)
OI vs no Injury	1.55 (1.08 - 2.22)	1.60 (1.10 - 2.31)	1.68 (1.15 - 2.44)	2.00 (1.29 - 3.09)
TBI vs OI	1.00 (0.60 - 1.65)	1.06 (0.64 - 1.77)	1.01 (0.60 - 1.69)	0.58 (0.32 - 1.06)
Omnibus p	0.004	0.002	0.001	0.003
	Cannabis [c]**			
n	n = 1649	n = 1649	n = 1649	n = 1649
TBI vs no Injury	1.71 (1.17 - 2.48)	1.64 (1.12 - 2.39)	1.64 (1.12 - 2.41)	1.14 (0.74 - 1.76)
OI vs no Injury	1.26 (0.89 - 1.78)	1.18 (0.83 - 1.68)	1.18 (0.83 - 1.69)	1.04 (0.69 - 1.58)
TBI vs OI	1.35 (0.84 - 2.19)	1.39 (0.85 - 2.26)	1.39 (0.85 - 2.27)	1.09 (0.62 - 1.92)
Omnibus p	0.035	0.094	0.092	0.702

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; * logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age and education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.35 Associations between traumatic brain injury from age 12 to age 16 years, with no additional orthopaedic injury, and substance use at age 17 years compared to orthopaedic injury on complete case sample

Substance Use	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Alcohol [a]*			
n	n = 1625	n = 1625	n = 1625	n = 1625
TBI only vs OI	2.07 (1.25 - 3.43)	2.13 (1.28 - 3.55)	2.09 (1.25 - 3.49)	2.41 (1.35 - 4.29)
Omnibus p	0.823	0.952	0.997	0.364
	Tobacco [b]**			
n	n = 1625	n = 1625	n = 1625	n = 1625
TBI only vs OI	0.83 (0.47 - 1.44)	0.89 (0.51 - 1.56)	0.84 (0.48 - 1.49)	0.59 (0.31 - 1.13)
Omnibus p	0.011	0.006	0.003	0.002
	Cannabis [c]**			
n	n = 1625	n = 1625	n = 1625	n = 1625
TBI only vs OI	1.16 (0.69 - 1.95)	1.21 (0.72 - 2.05)	1.21 (0.71 - 2.06)	1.12 (0.61 - 2.03)
Omnibus p	0.081	0.164	0.163	0.714

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; * logistic regression; **generalised ordinal regression; [a] alcohol measured using the Alcohol Use Disorder Identification Test (AUDIT); [b] tobacco measured using the Fagerström Test for Nicotine Dependence; [c] cannabis measured using the Cannabis Abuse Screening Test.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Model 3: As Model 2 with additional adjustment for substance use and crime variables

Appendix 3.36 Associations between traumatic brain injury and orthopaedic injuries from age 12 to 16 years and criminal behaviour at age 17 years on complete case sample

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 1681	n = 1681	n = 1681	n = 1681
TBI vs no Injury	2.22 (1.49 - 3.30)	2.02 (1.35 - 3.02)	2.01 (1.34 - 3.01)	1.52 (0.97 - 2.39)
OI vs no Injury	2.01 (1.40 - 2.88)	1.59 (1.10 - 2.30)	1.59 (1.10 - 2.30)	1.49 (0.99 - 2.24)
TBI vs OI	1.10 (0.67 - 1.81)	1.27 (0.76 - 2.10)	1.27 (0.76 - 2.11)	1.02 (0.58 - 1.79)
Omnibus p	<0.001	0.001	0.001	0.022
	Trouble with the Police [b]**			
n	n = 1651	n = 1651	n = 1651	n = 1651
TBI vs no Injury	1.89 (1.22 – 2.94)	1.75 (1.11 - 2.76)	1.74 (1.10 - 2.76)	1.21 (0.72 - 2.03)
OI vs no Injury	1.26 (0.81 - 1.95)	0.90 (0.57 - 1.42)	0.91 (0.57 - 1.43)	0.86 (0.52 - 1.42)
TBI vs OI	1.51 (0.84 - 2.70)	1.94 (1.06 - 3.55)	1.92 (1.05 - 3.53)	1.41 (0.72 - 2.77)
Omnibus p	0.063	0.751	0.728	0.756

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.37 Associations between traumatic brain injury from age 12 to age 16 years, with no additional orthopaedic injury, and criminal behaviour at age 17 years compared to orthopaedic injury on complete case sample

Criminal Behaviour	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
	Offences [a]**			
n	n = 1657	n = 1657	n = 1657	n = 1657
TBI only vs OI	0.87 (0.50 - 1.50)	1.03 (0.59 - 1.79)	1.03 (0.59 - 1.80)	0.85 (0.46 - 1.58)
Omnibus p	<0.001	0.004	0.004	0.041
	Trouble with the Police [b]**			
n	n = 1627	n = 1627	n = 1627	n = 1627
TBI only vs OI	0.91 (0.47 - 1.79)	1.20 (0.60 - 2.41)	1.19 (0.59 - 2.38)	0.92 (0.43 - 1.99)
Omnibus p	0.277	0.744	0.767	0.435

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; **generalised ordinal regression; [a] offences measured by self-report questionnaire at age 17 years; [b] trouble with the police measured by self-report questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)
Model 3: As Model 2 with additional adjustment for substance use variables

Appendix 3.38 Associations between traumatic brain injury and orthopaedic injuries from age 12 to 16 years and psychiatric symptoms based on the Strengths and Difficulties Questionnaire at age 17 years on complete case sample

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 3536	n = 3536	n = 3536
TBI vs no Injury	1.31 (0.77 – 2.24)	1.41 (0.83 – 2.41)	1.39 (0.81 - 2.39)
OI vs no Injury	1.35 (0.83 - 2.19)	1.37 (0.84 – 2.24)	1.37 (0.84 - 2.24)
TBI vs OI	0.97 (0.49 - 1.91)	1.03 (0.52 - 2.03)	1.02 (0.51 - 2.02)
Omnibus p	0.147	0.116	0.126
	Peer Problems [b]*		
n	n = 3526	n = 3526	n = 3526
TBI vs no Injury	0.76 (0.44 – 1.30)	0.71 (0.41 – 1.22)	0.72 (0.42 - 1.24)
OI vs no Injury	0.62 (0.36 - 1.06)	0.55 (0.32 - 0.94)	0.55 (0.32 - 0.95)
TBI vs OI	1.22 (0.59 - 2.55)	1.29 (0.62 - 2.70)	1.30 (0.62 - 2.74)
Omnibus p	0.053	0.015	0.018

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)

Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 3.39 Associations between traumatic brain injury from age 12 to age 16 years, with no additional orthopaedic injury, and psychiatric symptoms at age 17 years compared to orthopaedic injury on complete case sample

SDQ	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
	Conduct problems [a]*		
n	n = 3490	n = 3490	n = 3490
TBI only vs OI	1.13 (0.56 - 2.25)	1.2 (0.60 - 2.42)	1.18 (0.58 - 2.38)
Omnibus p	0.111	0.088	0.096
	Peer Problems [b]*		
n	n = 3480	n = 3480	n = 3480
TBI only vs OI	1.41 (0.66 - 2.98)	1.51 (0.71 - 3.21)	1.51 (0.71 - 3.22)
Omnibus p	0.079	0.025	0.029

Complete cases had no missing data for the exposure, outcome or covariates. TBI: traumatic brain injury; OI: orthopaedic injury; *logistic regression; [a] conduct problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years; [b] peer problems based on parent-completed Strengths and Difficulties Questionnaire at age 17 years.

Unadjusted: Injuries from birth to age 16 years with main substance use variable in each analysis
Model 1: As unadjusted with additional adjustment for pre-birth confounders (mother's age at birth, mother's education at birth, social class and gender)
Model 2: As Model 1 with additional adjustment for childhood confounders (early life events, parental bonding, positive and negative parenting experiences, maternal alcohol use and maternal tobacco smoking)

Appendix 5.1 Semi-structured interview for mTBI history

In this interview I am going to ask you about injuries to your head that may have occurred while you were playing rugby/hockey/other sport or in general life. Specifically, I'll ask about any concussions you may have sustained, it can be hard to remember these things but just try to be as accurate as possible. Concussion is a blow/knock to the head followed by a number of symptoms including:

- Loss of consciousness/getting knocked out
- Feeling dazed, confused or disoriented
- Loss of balance
- Blurred vision or seeing stars
- Feeling in a fog or slowed down
- Having memory problems
- Poor concentration
- Nausea or throwing up

Have you ever sustained a concussion or more serious head injury in your lifetime? Y / N. How many times? _

Have you ever sustained a concussion while playing or training for rugby? Y / N. How many times __

	Injury 1	Injury 2	Injury 3
Age it occurred			
Sport			
Training or during a match			
Activity other than sport			
Were you under the influence of alcohol or other substances?			
LOC (Duration)			
Other symptoms			
Confusion/disorientation			
Feeling in a fog			
Memory problems			
Nausea/throwing up			
Other			
Reported the injury to someone (if no, why not)			
Evaluated, by whom			
Continue to play			